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CONTENTS

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CANCER RESEARCH
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Malignant Tumors of the Uterus and Vagina in Untreated Mice of the PM Stock

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(Received for publication January 12, 1948)

Spontaneous malignant epithelial tumors of the uterus have been observed in rabbits but they occur rarely in mice (10). Aside from 4 sarcomas, Slye (8, 9) recorded 1 squamous cell carcinoma of the uterus, and Woglom (13) described one carcinoma of the uterus with metastases to the liver. Two hybrid mice with carcinoma simplex of the uterus have been observed in the Jackson Memorial Laboratory (11). Mice of several inbred strains, and hybrid mice, have been used in this laboratory to study the effects of steroid hormones on the origin of tumors of the uterine cervix (1, 3). Until recently no malignant epithelial uterine, uterine cervical, or vaginal tumors had been observed in untreated mice of the several inbred strains (A, C3H, CBA, JK, C12I, and C57), although mice of all strains that tolerated treatment with estrogen for long periods acquired carcinomas of the uterine cervix or vaginal fornices. While these studies were in progress a stock of mice became available in which the incidence of uterine cervical and vaginal tumors was quite high, even in untreated animals.

MATERIALS AND METHODS

The pair of mice from which all of the animals used in this investigation descended were obtained from Dr. H. B. Andervont who had in turn obtained the stock from the laboratory of Pybus and Miller (5, 6, 7) in England. The mice were reportedly from a strain that showed a high incidence of osteogenic sarcomas. Fifty-six untreated female mice of this stock, all but one of which were reared in our laboratory, were used as breeding animals and lived 200 days or longer. The stock was maintained by brother-to-sister matings for 10 generations. All the mice were kept under standard conditions, and on a diet of Purina Fox Chow and water *ad libitum,* with biweekly supplements of lettuce. Although inspected each day some of the animals died several hours before necropsy but most of them were killed when their general condition had declined or when tumors were observed. The autopsies were complete and tissues or organs that showed gross abnormalities were preserved in Bouin's fixative and stained in triosin and hematoxylin after sectioning. In addition several primary tumors and probable metastatic growths were stained with Laidlaw's connective tissue stain in order to detect reticular fibers.

OBSERVATIONS

Thirteen of the 56 mice had uterine or vaginal tumors at the time of death. The mice with tumors of the genital tract survived on the average of about one month longer than those without such tumors (Table I).

TABLE I: THE SURVIVAL TIME OF UNTREATED FEMALE MICE OF PM STOCK

Mean age at death	Days
All mice	534
Without tumors of genital tract	528
With tumors of genital tract	562

A summary of the morphological observations is given in Table II. Detailed descriptions of all the tumorous animals will not be given, but a few interesting cases are cited for illustration.

One animal (No. 49) was killed in poor general condition at 625 days of age. At autopsy the entire vaginal wall was thickened, in places nodular or papillary in appearance (Fig. 1). The uterine cornua revealed nothing of note. A small grey-white nodule was seen in the upper lobe of the right lung. The adrenals were larger than usual but were smooth.

* This investigation has been supported by grants from The Jane Coffin Childs Fund for Medical Research, The Anna Fuller Fund and the National Cancer Institute (U.S. P.H.S.).

** Anna Fuller Fund Fellow.

TABLE II: A SUMMARY OF THE MALIGNANT TUMORS IN MICE OF THE PM STOCK

No.	Age	Location	Type of cell	Malignancy		Lesions in other organs						
				Mitoses*	Local invasion	Liver	Ovary	Adrenal	Kidney	Lymph node	Elsewhere	Diagnosis
6	571	Vagina	Epidermoid	+++	+							Epidermoid carcinoma
47	623	Vagina and cervix	"	+++	+		+*	+*				"
49	625	"	"	+++	+			+*			Lung	"
61	488	"	"	+++	+†	+‡			+	+†		"
74	702	Cervix	"	+++	+					−		"
57	548	Uterine horns	Undifferentiated cell	++	+	+	+				Mesentery	Undifferentiated cell carcinoma
58	643	Cervix	"	++	+			+				"
63	424	"	"	++	+	+	+	+	+	+		"
69	594	Horns	"	++	+	+	−		+			"
80	546	Right horn	"	++	+							"
83	613	Cervix	"	++	+		−	−				"
93	474	Cervix and horns	"	++	+	+	+	−				"
91	418	Horns	Spindle cell	++	+	+	+	+			Pancreas and stomach	Spindle-cell sarcoma

* Estimated according to the mitoses seen at high power magnification by microscopic examination.
† Lesions in these sites were of undifferentiated cells.
‡ Lesions in the liver differed from those in the uterine tumor.

Microscopically serial sections from the cervix and vagina indicated that the tumor arose in the fornix of the vagina and had extended both cranially and caudally. A part of the longitudinally folded, stratified epithelium of the fornices and vagina remained intact but was unusually hyperplastic. The tumor consisted principally of confluent papillary growths of well differentiated epidermoid epithelial cells (Fig. 4). At the bases of the larger epidermoid growths were areas of large, irregular and anaplastic cells (Fig. 3), some of which were spindle-shaped and appeared to be exceptionally invasive (Fig. 2). The nuclei were large and hyperchromatic and many were in mitoses. In some areas small stands of tumor cells invaded the muscle fibers, extended into the lymphatics (Fig. 3), blood vessels (Fig. 4) and along the perineurium (Fig. 5). The stroma was scanty and showed a moderate and irregular leukocytic infiltration. Congestion and edema were also observed. The tumor extended to involve the parametrium and the surrounding tissue.

Several nodules of metastatic tumor cells were found in sections of the right lung. The metastases were composed of closely-packed polygonal cells, some of which assumed a glandular arrangement and had well defined cellular boundaries and hyperchromatic nuclei, and presented the characteristics of epidermoid cells.

In the adrenals small collections of tumor cells in mitoses were observed in the reticular zone accompanied by a great number of polymorphonuclear leukocytes.

A tumor of the uterine cervix was found in a mouse (No. 61), killed when 488 days old, a daughter of the mouse mentioned above. At autopsy the uterine cervix was enlarged, and the walls of the vagina and cervix were thickened. A large polypous mass arose from the posterior aspect of the cervix and vagina (Fig. 6). Both uterine horns were small and atrophic. The liver was large and contained many grey white nodules. The lumbar lymph nodes were slightly larger than normal.

Histologically the tumor consisted mainly of well differentiated squamous cells arranged to form papillary projections in some places. The cells in the deep border of the tumor were anaplastic; they invaded the muscle fibers and the blood vessels. In some areas near the base of the polypous growth small collections of spindle-shaped cells were scattered rather diffusely and on close examination were continuous with the basal layer of the neoplastic

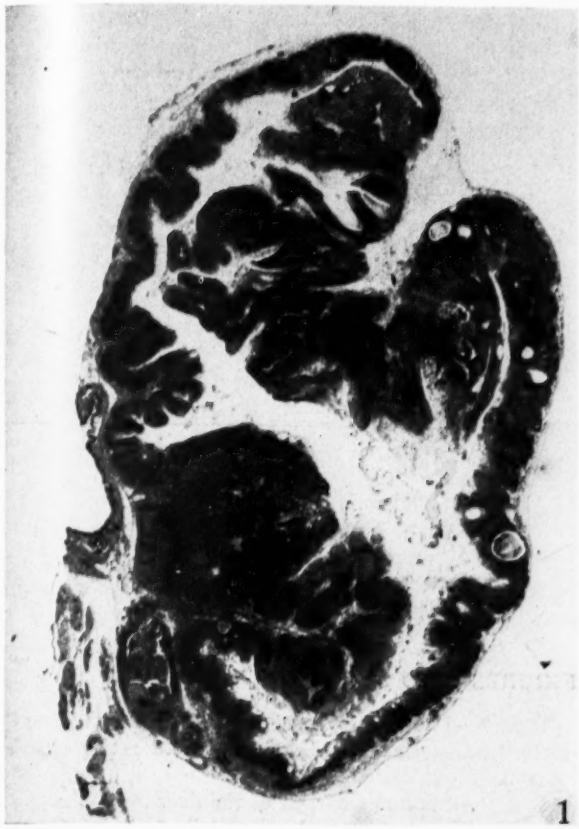
DESCRIPTION OF FIGURES 1 TO 4

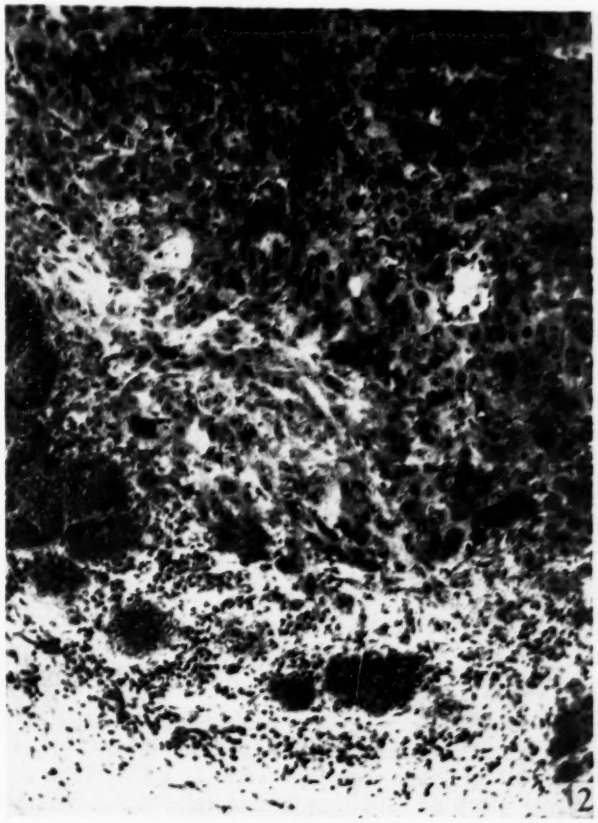
FIG. 1.—Section through upper vagina of mouse (No. 49) showing the papillary appearance of tumor and non-tumorous vaginal mucosa. Mag. × 16.

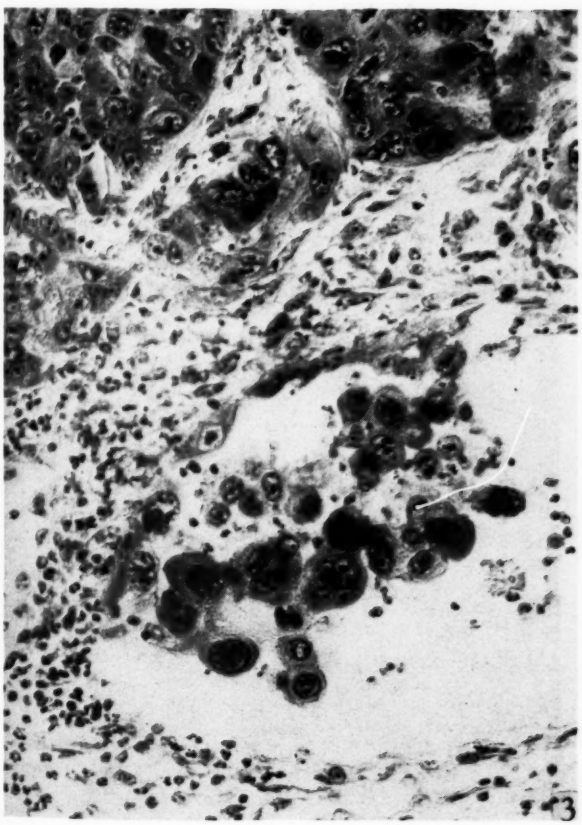
FIG. 2.—Photomicrograph of same tumor showing lack of polarity and spindle-cell transformation of tumor cells at base of papillary growth. Mag. × 100.

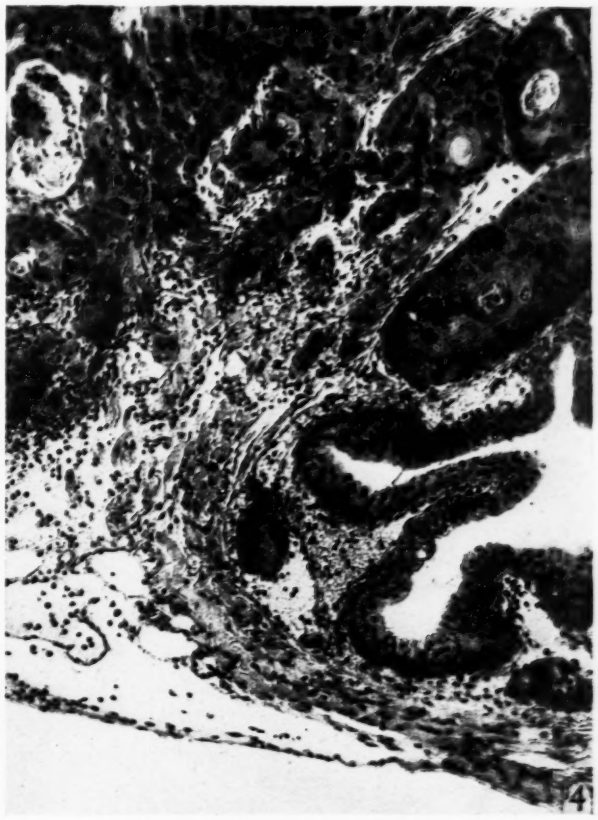
FIG. 3.—Same tumor revealing lymphatic spread of neoplastic cells. The lymphatic in lower part of photograph contains group of malignant cells. Mag. × 200.

FIG. 4.—Photomicrograph of section of same tumor showing peripheral portion of carcinoma with group of tumor cells in blood vessel. Non-tumorous vaginal epithelium is shown at lower right. Mag. × 100.









Figs. 1-4

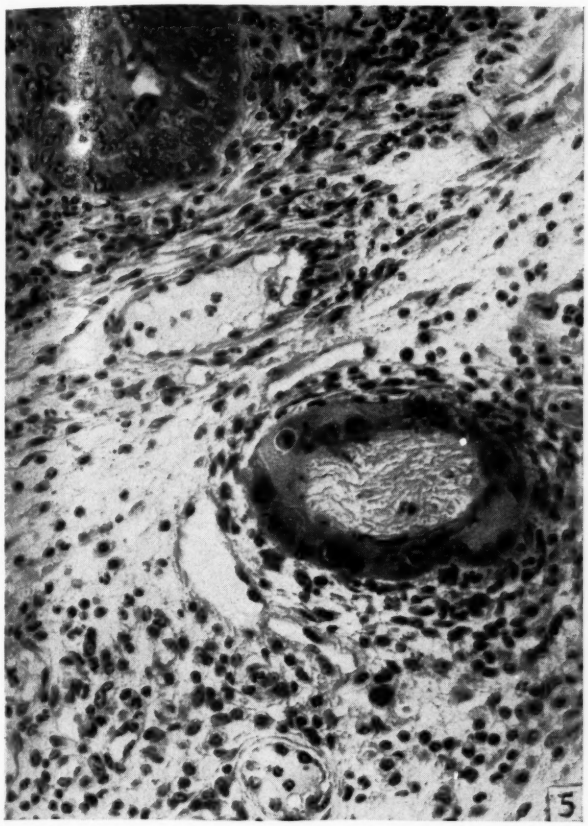
DESCRIPTION OF FIGURES 5 TO 8

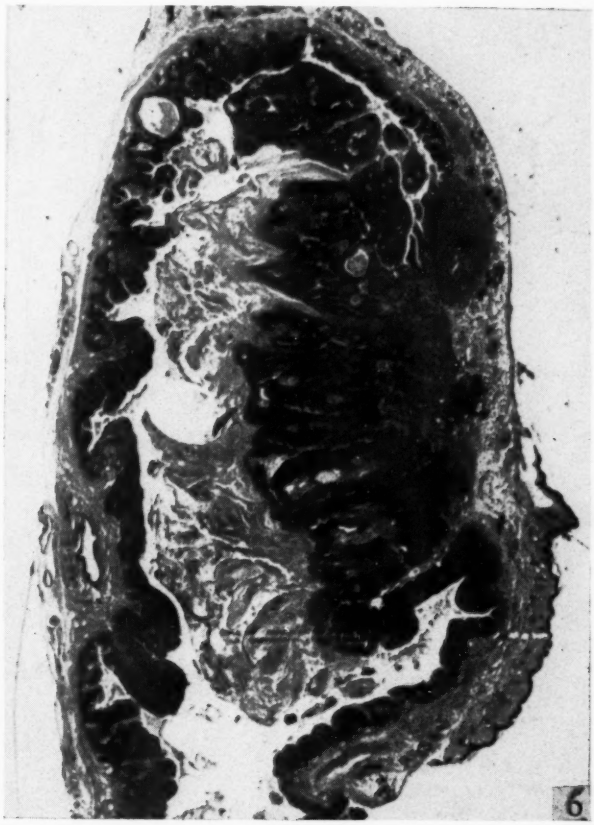
FIG. 5.—Photomicrograph of same tumor showing perineural spread of tumor cells. Mag. × 200.

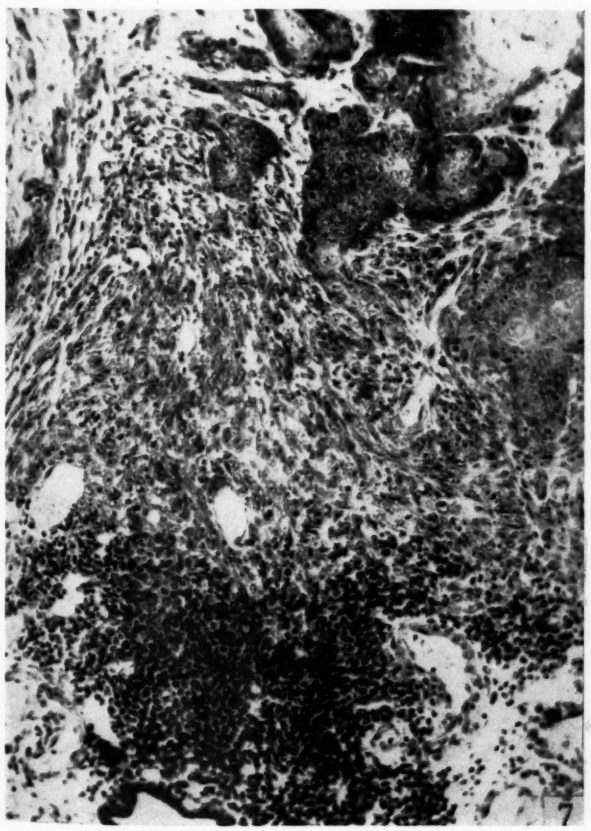
FIG. 6.—A section from the cervix of mouse (No. 61) showing polypous carcinoma arising from dorsal wall of uterine cervix. Mag. × 16.

FIG. 7.—Photomicrograph of same tumor showing spindle-cell transformation of the epidermoid cancer cells. Mag. × 100.

FIG. 8.—Photomicrograph of the liver of mouse (No. 61) showing diffusely scattered tumor cells replacing the parenchyma. Mag. × 100.







Figs. 5-8

epithelium (Fig. 7). In other areas acinar structures lined by either a hyperplastic squamous epithelium or a low stratified epithelium with a surface layer of columnar mucous cells were observed. The entire vaginal epithelium was hyperplastic and folded. The scanty stroma contained occasional mononuclear and plasma cells in the deeper areas.

The architecture of the liver was obliterated and the hepatic tissue was extensively replaced by irregular sheets of cells varying greatly in shape and size. Some of them were oval and some of them were spindle-shaped. They were scattered diffusely throughout the hepatic tissue without definite alveolar arrangement (Fig. 8). Many irregular sheets of tumor cells grew into the central and hepatic veins. Many of the hepatic cells showed fatty change. Small groups of round and undifferentiated cancerous cells were observed in the lumbar lymph nodes. Some glomerular tufts of the kidneys contained small collections of tumor cells, some in mitoses. The cytoplasm of the cells lining the convoluted tubules was granular and some tubules contained red blood cells.

The vagina of a third mouse (No. 74) that was killed when 702 days old, was large and irregular. The dorsal lip of the cervix was irregular and protruded into the vaginal cavity. The left ovary was slightly enlarged.

Histological examination of the uterus and vagina revealed that the tumor arose from the cervix and extended to involve the outer coat of the uterine horns. The tumor was composed mainly of epithelial cells that were well differentiated in some places but lacked polarity and were polygonal or spindle-shaped in other areas. The cells that penetrated the basement membrane had lost the appearance of basal cells, were smaller and irregular and merged into the stroma; many mitotic figures were observed in such areas. Marked proliferation of the fibrous stroma with leukocytic infiltration was present.

At autopsy the abdominal cavity of another mouse (No. 57) was filled with bloody fluid. Arising from the upper part of the left uterine horn was a mass, measuring 1.5 cm. in diameter. Both ovaries were large. The liver was extremely large and mottled. The tumor on the left horn was composed of oval and spindle cells with a small amount of stroma. The cells had little cytoplasm but had large and hyperchromatic nuclei. Mitoses were frequent. No definite pattern of cellular arrangement was noted in some areas, but the cells were arranged in alveolar masses in other regions (Fig. 9). Preparations stained by Laidlaw's method revealed no reticular fibers between individual tumor cells (Fig. 10). Some remnants of the myometrium persisted and were dispersed by the neoplastic growth. Portions of the tumor had become necrotic, showed spaces that had been apparently occupied by soluble fusiform crystals, and contained some giant cells. The tumor had invaded the entire length of the uterine horn.

The nodular growths in the liver were composed of irregular sheets of undifferentiated cells arranged in an indefinite alveolar pattern, and identical with those seen in the uterus (Fig. 11). Although reticulum fibers were abundant they were lacking between individual tumor cells (Fig. 12). The ovaries also were almost entirely replaced by tumor tissue; only a few ovarian follicles remained.

Mouse No. 58 when killed at 643 days of age had a vagina larger than usual and pale grey in color. The cervix was also enlarged and a tumor growth approximately 2 cm. in greatest diameter was attached to the posterior pelvic and abdominal wall. Both uterine horns contained clear brown fluid. The lumbar lymph node was slightly enlarged. The kidneys were large and pale. The liver and pancreas contained many pale grey nodules.

Histologically a part of the cervical mucosa was replaced by a tumor composed of a mixture of oval, polygonal, and spindle cells, that varied greatly in size. Mitoses were frequent. In some areas the tumor cells were arranged in rosettes or palisades and the fibrous stroma was inconspicuous. The remaining epithelium of the upper vagina was of a low stratified type, the superficial cells of which contained a large amount of mucoid material. The ovaries showed no change not commonly found in old mice. The reticular zone of the adrenal contained some hyperchromatic cells in mitoses together with a number of polymorphonuclear leukocytes.

Marked anasarca was noted during autopsy in mouse No. 63. The uterine cervix was replaced by a tumor measuring approximately 1.5 cm. in diameter (Fig. 13). It was whitish and moderately solid. The liver was studded with many pale grey spots.

On histological examination the tumor was composed of polymorphic cells in alveolar arrangement; the cells resembled those described above for mouse No. 58. The central part of the tumor was necrotic. Peripherally it had invaded the muscle, parametrium and also the surrounding tissue. Massive metastatic growths were found in the liver (Fig. 14). The alveolar pattern of the neoplastic cells, both in the uterus and in the liver, was brought

out by the Laidlaw's silver impregnation method for reticulum (Fig. 15). The capsule of a lumbar lymph node was partly encircled by the tumor tissue and at one place invaded by irregular sheets of cells packed closely together.

Another mouse (No. 69), a daughter of mouse No. 63, also had marked anasarca at the time of death. The vagina ended without perforating the skin and both the vagina and uterine horns were distended with brownish fluid. The uterine wall was thickened in places and contained several white nodular masses (Fig. 16). Both ovaries were larger than usual; the liver was large and contained many grey-white nodules; and both kidneys were pale.

Histologically the tumors of the uterine wall consisted of closely packed undifferentiated cells, rather uniform in size, but they differed greatly in shape. The nontumorous areas of the uterine wall were extremely thin and the lumen contained eosinophilic material. Throughout the liver were diffusely scattered irregular sheets of cells similar to those in the uterine tumor. Metastatic nodules were also observed in the perirenal tissue.

The abdomen of another mouse (No. 91, killed when 418 days old) contained a small amount of serous fluid. Both uterine horns were large and distended with bloody fluid. The uterine cervix and right horn were occupied by a grey-white and nodular mass. The vagina was thin-walled. Many grey-white nodules were found over the peritoneal surfaces of the abdominal wall and diaphragm. The liver contained grey-white patches. The stomach was thick-walled and adherent to the surrounding organs and parietes. The capsules of the ovaries were of grey-white color and the adrenal glands were enlarged.

Microscopically, with the exception of a small portion of mucosa, the uterine wall and broad ligaments were destroyed and replaced by a tumor composed of interlacing bundles of spindle cells. Necrosis and ulceration were observed in some areas. The epithelium of the uterus was moderately hyperplastic and in places showed squamous metaplasia. Metastatic growths were found in the periadrenal regions, in the pancreas, liver and in the wall of the stomach and were histologically similar to the uterine tumor.

DISCUSSION

Of the 13 tumors of the genital tract observed in the 56 female mice of the PM stock 5 were epidermoid or squamous cell carcinomas. One tumor apparently arose from the distal vagina, one from the uterine cervix, and the others from either the uterine cervix or upper vagina. Seven of the tumors were composed of undifferentiated cells and arose from the uterine cervix or cornua. One tumor was a spindle-cell sarcoma.

All tumors were malignant as indicated by the lack of cellular polarity, increased number of mitoses, local invasion and distant metastases. The epidermoid carcinomas possessed all the morphological criteria common to carcinomas of the uterine cervix in man. In the undifferentiated cell group the morphology of the tumor cells varied slightly, some resembling fibrosarcomas or myosarcomas and others neurofibrosarcomas; but most tumors exhibited a rather definite or incomplete alveolar pattern that was well illustrated by silver impregnation in the two cases so stained. Local invasion was observed in all. Tumor cells in the muscle fibers, lymphatics, blood vessels and along the nerve sheaths were demonstrable in animals with the epidermoid tumors whereas peritoneal extension to involve the broad ligament, ovaries, periadrenal and perirenal tissues, the stomach and pancreas was more frequently encountered in the undifferentiated cell tumors. Five mice with tumors had associated lesions in the liver. In all except one animal the lesions in the liver were multiple, histologically identical with the primary tumors, and were considered to be secondary tumors. In one instance (No. 61) the lesions in the liver differed histologically from the cervical or upper vaginal carcinoma; it is not certain whether the hepatic lesions were profound stromal reactions or an unrelated tumor. The lymph nodes were involved in two cases and the lungs were involved once. The lesions in these two sites were of less differentiated cells but the evidence of lymphatic and hematogenous spreads of the tumor strongly suggests their secondary nature.

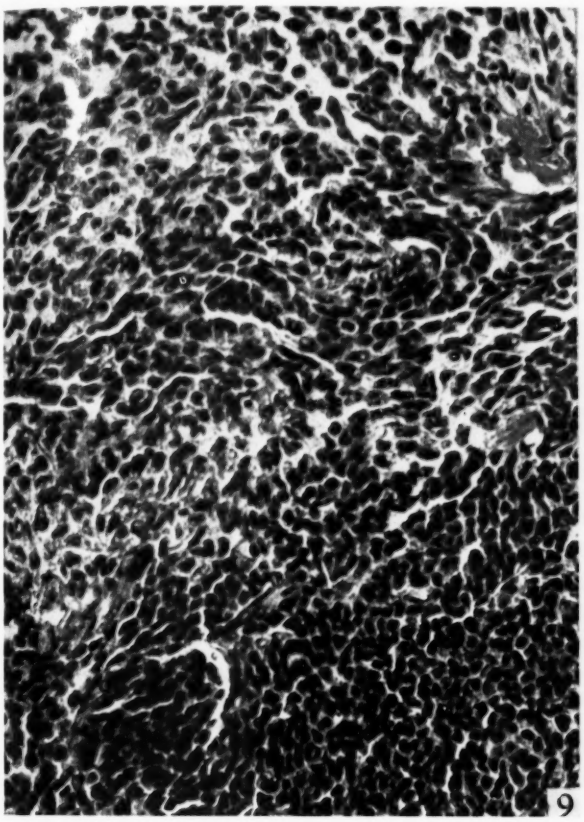
The presence of multiple types of cells in the epidermoid carcinomas was observed in several instances. The cells in the undifferentiated tumors

DESCRIPTION OF FIGURES 9 TO 12

FIG. 9.—Photomicrograph of uterine tumor of mouse (No. 57) showing definite alveolar pattern of the undifferentiated tumor cells. Mag. × 200.

FIG. 10.—Photomicrograph of the same tumor (Laidlaw's stain) showing the lack of reticulum between individual tumor cells. Mag. × 200.

FIGS. 11 and 12.—Photomicrographs of the liver of mouse No. 57 showing the metastatic growth in the liver. Hematoxylin and triosin stain (Fig. 11) and Laidlaw's stain (Fig. 12). Mag. × 200.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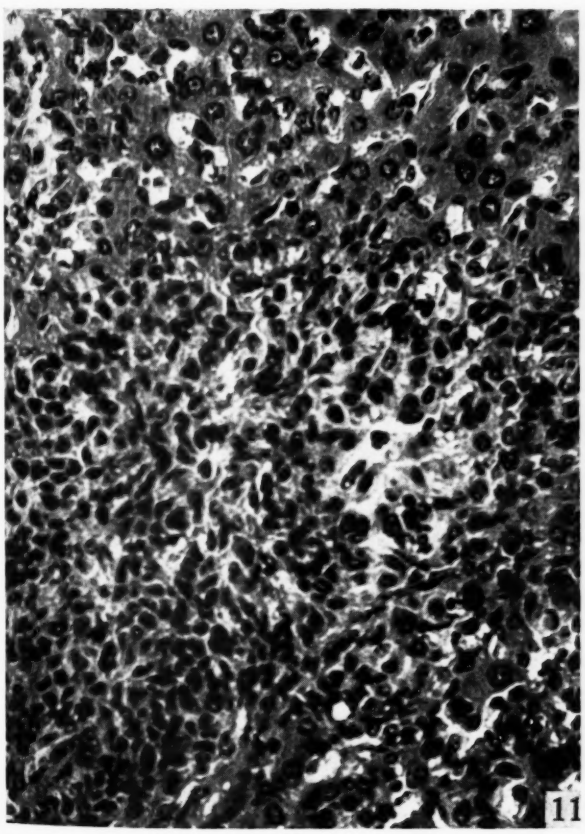
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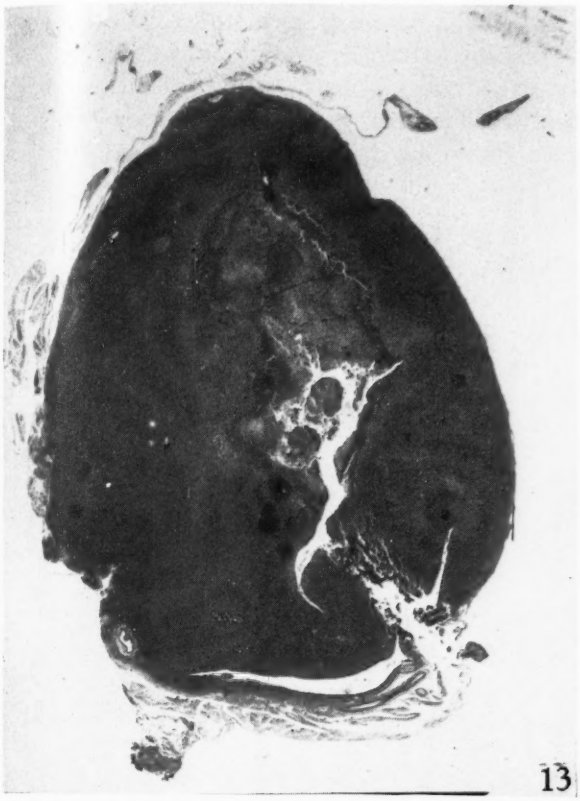
Figs. 9-12

DESCRIPTION OF FIGURES 13 TO 16

FIG. 13.—A section of tumor growth in uterine cervix of mouse No. 63. Slit-like opening at lower part of photograph is the vagina.

FIGS. 14 and 15.—Liver metastases of mouse No. 63 revealing definite alveolar pattern of the tumor cells. Hematoxylin and triosin. (Fig. 14) and Laidlaw's silver impregnation (Fig. 15). Mag. × 100.

FIG. 16.—Photograph of mouse (No. 69) revealing markedly distended uterine horns with several nodular masses. The ovaries were also enlarged and replaced by tumor cells. The entire vagina, as well as the uterine cornua, was distended down to its blind subcutaneous termination.



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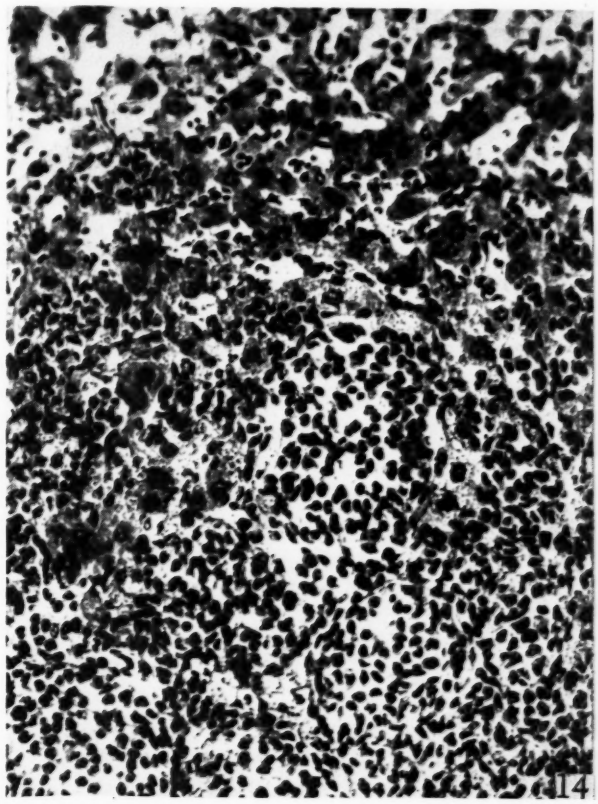

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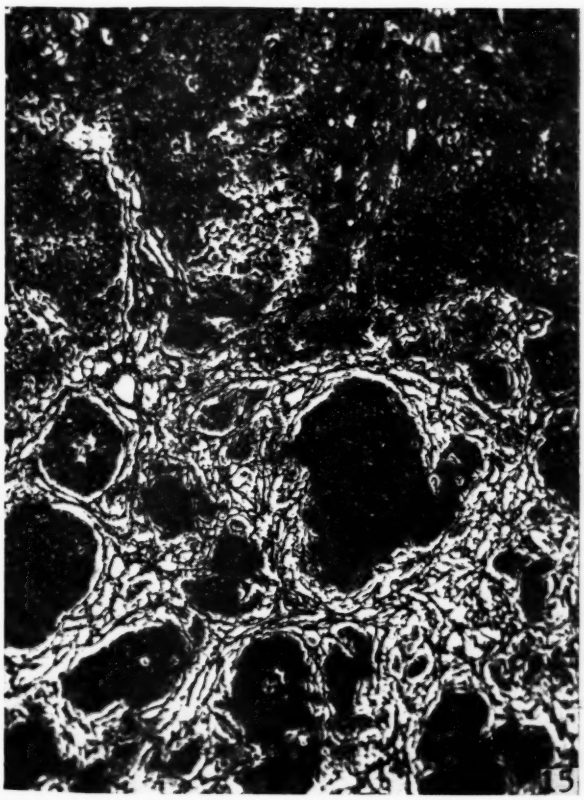

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Figs. 13-16

were probably of epithelial origin judging by the apparent transformation of the squamous cell into spindle or undifferentiated types of cells in some instances and the absence of reticulum between the individual tumor cells in others. The marked stromal proliferation associated with the undifferentiated cell carcinomas has led some of the observers to believe that they were carcinosarcomas or sarcomas. Further study is needed to clarify this problem.

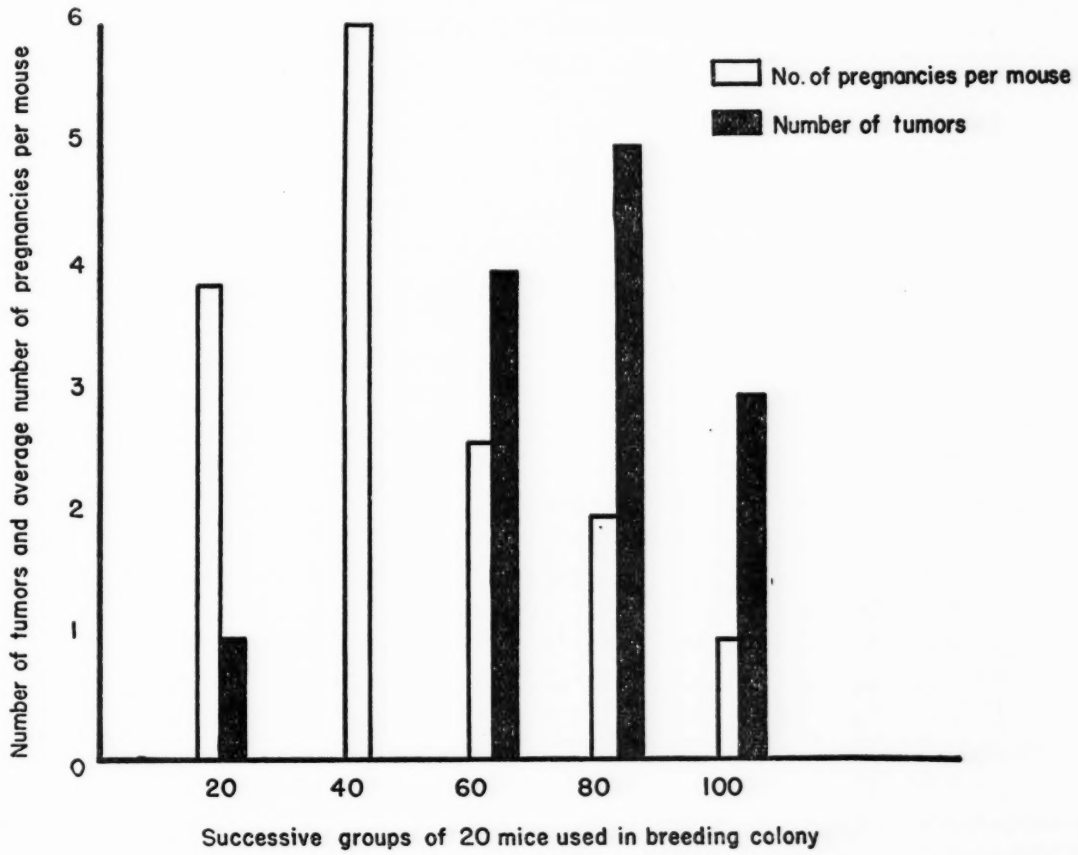

FIG. 17.—Reproductive history of mice of the "PM" stock. A total of 106 mice, both male and female, were used as breeding stock.

The high incidence of tumors of the female genital organs of mice of this special line is unique. Carcinomas of the uterine cervix have never been observed in our laboratory (3) in untreated mice of other strains and rarely in other laboratories (5-12). One precancerous lesion of the vaginal epithelium was described by Suntzeff and associates (12). Tumors of the uterus, the uterine cervix and upper vagina have not occurred with unusual frequency in mice of any strain although Slye (9) noted a tendency for them to occur more frequently in one family of her animals. The high incidence of such tumors in mice of the PM stock indicates the existence of either (a) a genetic tendency for such tumors, or (b) a genetic tendency for a condition that predisposes to such tumors. Genetically this stock of mice originated from mice of the Simpson strain that at one time showed a high incidence of mammary adenocarcinomas (5). The incidence of mammary tumors in the line of mice from which the PM stock was derived was very low, and none have been observed in our laboratory, but the incidence of sarcomas was high, especially osteogenic sarcomas (6, 7). Osteogenic tumors likewise have not been observed in mice of the PM stock in our laboratory. The estrogen-treated mice of this stock showed a high incidence of lymphoid tumors (4) and a low tolerance to prolonged treatment with large doses of estrogen. Furthermore the femurs of estrogen-treated mice of the PM stock show unique responses to estrogen (2).

The low fertility of the PM stock was a striking feature. Many pregnancies were recorded (as determined by the general appearance of the animals), but the young were either born dead, died shortly after birth or were not observed (Fig. 17). Be-

cause the animals were examined but once each day, young might have been born and destroyed without detection. Two of the mice were sterile; they had imperforate vaginas and one of them had multiple tumors of the uterine horns. The fecundity of the mice decreased with increased generations of inbreeding, and the incidence of genital tumors increased concomitantly (Fig. 18). The strain was lost after it had been carried for 11 generations in the laboratory but not before it had been outcrossed and one or two back-cross generations obtained.

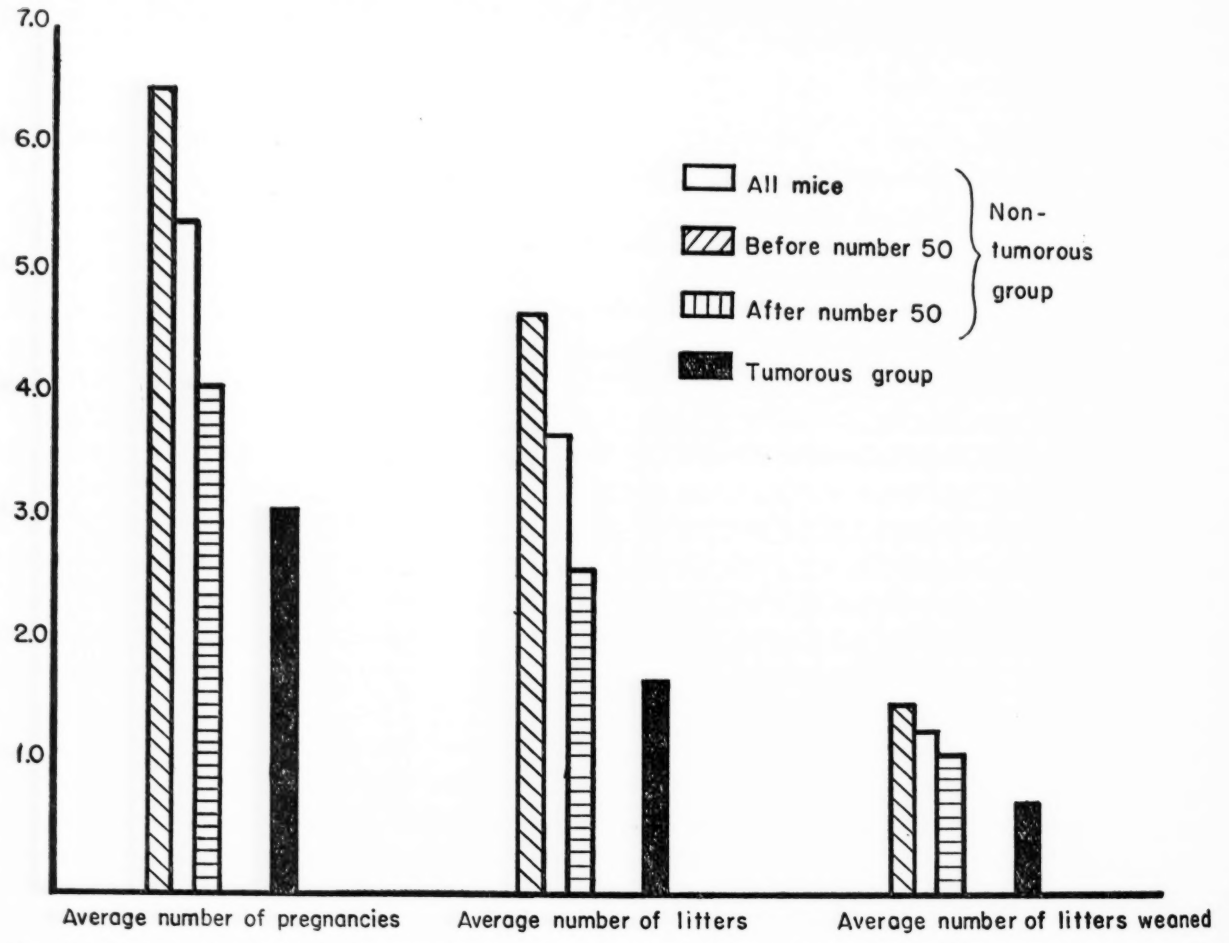

FIG. 18.—Reproductive history of successive groups of mice of "PM" stock and number of tumors per group. (Only 56 female mice of entire group survived 200 days or more, other animals were males or females that survived less than 200 days.)

Male mice of the PM stock were fertile, at least when mated with mice of the C3H strain; usual numbers of viable young were born and reared. The male mice, however, differed from male mice of other strains in that many of them had few mammary rudiments and rarely were more than two or three mammary rudiments present. The tendency for imperforate vagina and for few mammary rudiments indicates that a hormonal imbalance may have existed during fetal life. Adequate studies were not made to prove this assumption, however.

The relationship of the several animals with the tumors of the female genital tract fails to give any significant data except insofar as the derivatives of animal No. 9 gave rise to no descendants that had such tumors. The descendants of this mouse dropped out after the fifth generation of inbreeding. Furthermore all of the offspring after the sixth generation were descendants of mice (No. 47 and 49) that had epithelial tumors of the genital tract. The tendency for the undifferentiated and epidermoid tumors to occur in mice of the same group and in direct descent indicates that similar etiological agents may be active in both circumstances.

SUMMARY

Thirteen tumors of the uterus or vagina appeared in 56 female mice of one stock. Five of the tumors

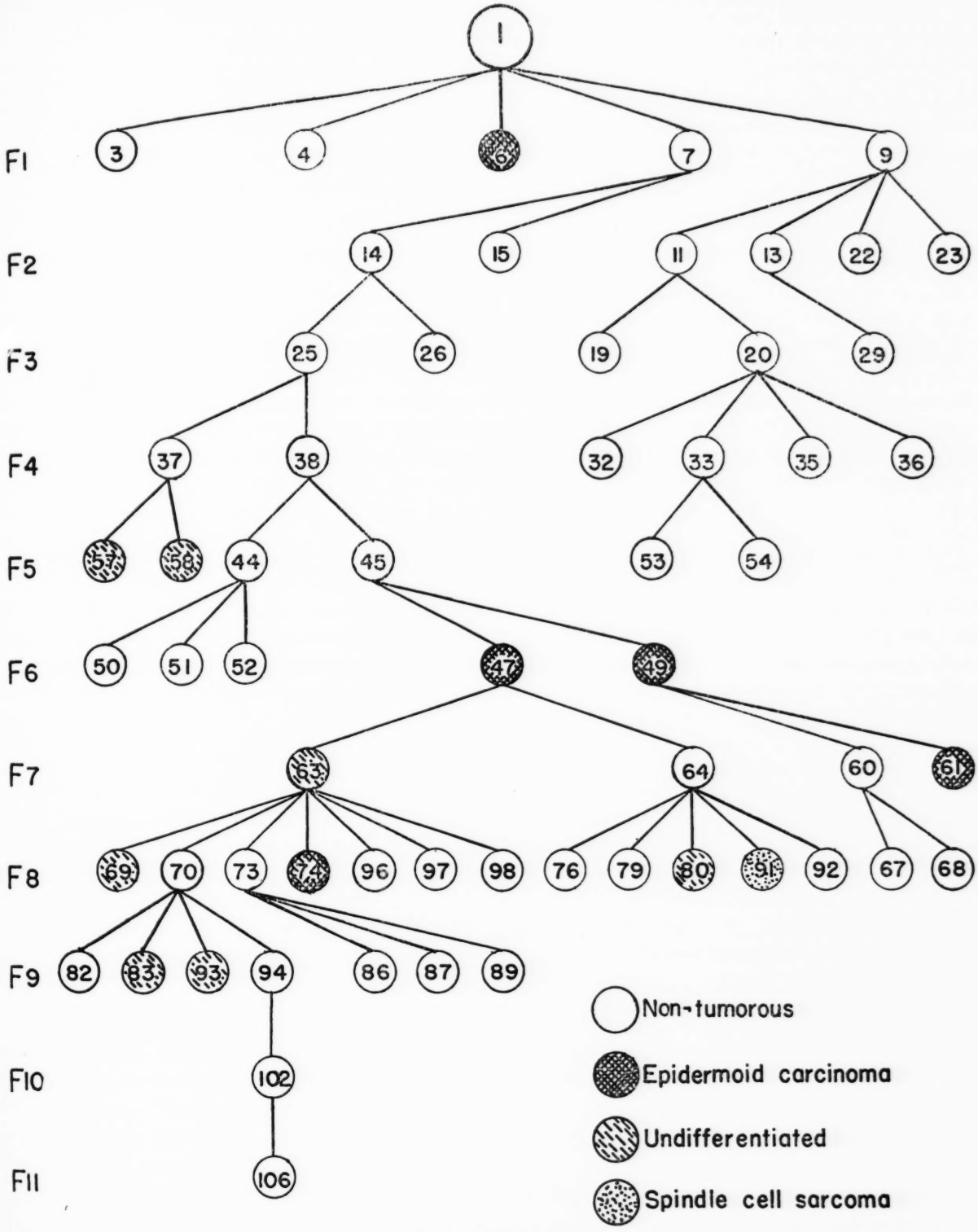

FIG. 19.—Lineage of mice of "PM" stock. Figures in circles are laboratory numbers of the animals.

were carcinomas of the vagina or uterine cervix; 7 were malignant tumors composed of undifferentiated cells, probably of epithelial origin, and one a spindle-cell sarcoma.

Mice of this stock showed low fertility, and two females had incompletely developed vaginas. The fertility decreased with continued inbreeding and the incidence of tumors increased.

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Influence of Thiouracil on Carcinoma Induced by 2-Acetaminofluorene*

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Bielschowsky (2) reported the occurrence of carcinoma in the rat's thyroid gland made hyperplastic by administration of allyl thiourea when the carcinogen, 2-acetaminofluorene (AAF) was administered simultaneously. Applying a similar principle, we investigated the effects of administration of this carcinogen to rats in which hyperplasia of target organs was produced by injection of male and female sex hormones. No evidence of malignancy was observed in these organs under these circumstances, but it was found that simultaneous administration of sex hormones and AAF enhanced the development of carcinoma of the liver (4). At the same time, experiments were conducted designed to repeat the work of Bielschowsky, with minor variations. His observation regarding the development of thyroid malignancy was confirmed. It was noted also that the liver of rats receiving thiouracil was protected against the carcinogenic effect of AAF.

The present communication reports (a) studies of the protective action of thiouracil against the hepatic lesions caused by AAF and (b) the effects on the thyroid gland of simultaneous administration of AAF and thiouracil. Inasmuch as thiouracil was found to protect the liver against carcinoma induced by AAF, and sex hormones enhanced the development of liver cancer, study of the protective action of thiouracil was extended to animals receiving AAF and sex hormones simultaneously. The effect of thiouracil on the development of liver cancer by *p*-dimethylaminoazobenzene was also investigated.

In an attempt to study the mechanism by which thiouracil exerts its protective action, the effect of methionine was investigated, inasmuch as it has been suggested that thiouracil protects against dietary cirrhosis by lowering the requirement for methionine (6). The possible role of hypothyroidism in this protective mechanism was studied in experiments on thyroidectomized rats and on rats receiving thiouracil and thyroxin simultaneously.

MATERIAL AND METHODS

The rats employed in this study were of the Sherman strain and Wistar descendants, weighing 75 to 100 gm. at the beginning of the experiment. They were given the carcinogenic diet and the various experimental supplements until death or sacrifice. AAF (0.03 per cent) was incorporated in a corn meal diet (16 per cent protein) reinforced by powdered brewers' yeast, as previously reported (4, 15). A motor-driven dough mixer was used to insure thorough mixture of the dietary ingredients. *p*-dimethylaminoazobenzene was given in a high-fat diet (20 per cent corn oil) described by Kline and his associates (7).

Thiouracil was administered in the drinking water in concentrations of 0.05 or 0.1 per cent.

Testosterone propionate was injected (in sesame oil) in the dosage of 0.5 mgm., three times weekly and estradiol dipropionate (in sesame oil), 10 gamma, three times weekly.

Methionine was dissolved in the drinking water. In order to insure complete intake, 40 mgm. were dissolved in 10 cc. of water and offered in the evening. During the following day, the empty drinking bottles were replaced by bottles containing tap water, which was, in the evening, again replaced by the methionine solution.

Crystalline l-thyroxine was dissolved in slightly alkalinized water and was injected subcutaneously daily in doses of 5 to 10 gamma.

The animals were killed by exsanguination under light ether anesthesia. Tissues were fixed in Bouin solution, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin, and, in some instances, with Masson's trichrome stain. Organ weights were taken rapidly on a Roller-Smith torsion balance, on fresh tissues freed from fat and connective tissue.

RESULTS

The findings in the various experimental groups are presented in Tables I to IV.

Effect of thiouracil on hepatic carcinoma (Table I).—The histologic characteristics of the hepatic lesions produced by AAF have been described elsewhere (4, 15). The aggravating influence in this connection of testosterone is not readily apparent from the figures included in the table. Because of the high incidence of hepatic carcinoma in male rats receiving AAF alone for more than 200 days, this influence is exhibited chiefly in increased in-

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cidence of cancer of the liver in males treated less than 200 days (58 per cent after AAF alone; 90 per cent in testosterone-treated) and in the greater average weight of the cancerous livers in the testosterone-treated animals (15). It is apparent that the protective influence of thiouracil against hepatic carcinoma induced by AAF is exerted also against the aggravating effect of testosterone. The incidence of carcinoma of the bladder, thyroid and ductus acusticus was not altered in males whereas that of mammary carcinoma in females was slightly but significantly decreased in the thiouracil-treated group.

Measurement of the food (and carcinogen) intake and increase in weight of the various experimental groups revealed no significant difference between the animals receiving AAF alone and those receiving also thiouracil.

Interesting differences were noted in the androgenic effects of testosterone in the rats receiving AAF with and without thiouracil. The AAF-treated group exhibited a relatively mild androgenic effect, as gauged by increase in prostatic weight (Table II) and inhibition of spermatogenesis. Mature sperms were present in the testes in 9 of 24 rats treated up to 254 days (Fig. 1). The androgenic response in the AAF-thiouracil group was essentially normal, the prostatic weight increasing considerably, as shown in Table II, and mature sperm appearing in the testes in only 2 of 40 animals (147 and 175 days respectively), the remainder exhibiting arrest of spermatogenesis (Fig. 2).

Cancer of the liver occurred in 15 of 17 (88 per cent) female Sherman rats receiving *p*-dimethylaminoazobenzene for 69 to 133 days and in 6 of 13 (46 per cent) receiving this carcinogen and thiouracil for 69 to 125 days. No difference was observed in males treated similarly.

Effect of methionine (Table III).—Administration of methionine had no influence upon the incidence nor the histologic characteristics of the hepatic neoplasms induced by AAF, with or without testosterone. However, the average weight of the cancerous livers in the methionine-treated group was lower than in those not receiving this supplement. One peculiar feature was a greater severity of cirrhotic changes in non-neoplastic portions of the liver in the methionine-treated animals as compared with those receiving only AAF.

Effect of thyroidectomy and of thyroxin (Table I).—None of the thyroidectomized rats survived for a long enough time to permit any conclusion regarding the influence of hypothyroidism *per se* upon the incidence of hepatic carinoma due to AAF.

Attempts to prevent the development of hypothyroidism in rats receiving thiouracil by simultaneous administration of thyroxin were not entirely successful. Despite the fact that the dosage employed (5 to 10 gamma daily) is adequate to prevent hyperplasia (gross and microscopic) of the thyroid of normal rats receiving thiouracil, it did not do so in this series receiving also AAF. No liver cancer developed in the few females that survived sufficiently long to permit them to be included in these

TABLE I: INFLUENCE OF THIOURACIL ADMINISTRATION ON CANCER OF THE LIVER PRODUCED BY 2-ACETAMINOFLUORENE

	AAF		AAF Thiouracil		AAF Testost. prop.	AAF Thiouracil Testost. prop.	AAF Thiouracil Thyroxine	
	male	female	male	female	male	male	male	female
Number of rats	55	54	18	12	24	17	25	8
Duration (days)	133–333	120–375	154–302	130–312	127–254	147–249	147–349	202–279
Liver Cancer	46 (83.6%)	19 (46%)	4 (22.2%)	—	20 (83.3%)	5 (29%)	18 (72%)	—
Liver weight,* non-cancerous livers	5.8	5.6	4.7	4.4	8.5	5.2	6.1	4.9
Liver weight,* Cancerous livers	12.1	13.0	7.4	—	15.4	10.2	9.2	—
Other cancers	7 (12.7%)	17 (30%)	4 (22.2%)	5 (41.6%)	3 (12.5%)	3 (17.6%)	3 (12%)	1 (12.5%)

* gm. per 100 gm. body weight

TABLE II: WEIGHT OF THE PROSTATE

Type of exper.	Number of animals	Prostatic weight (mgm. per 100 gm. body wt.)	Difference	"P" of difference
1. AAF	13	118		
			(2) − (1) = 48	>0.1
2. AAF Thiouracil	7	166		
3. AAF Testost. prop.	12	304		
			(4) − (3) = 329	<0.01
4. AAF Testost. prop. Thiouracil	10	633		

statistics. In the males, the addition of thyroxine apparently overcame, to a considerable degree, the protection afforded by thiouracil against AAF-induced hepatic carcinoma.

Thyroid lesions.—In Table IV are presented data regarding the incidence of thyroid adenoma and carcinoma in rats receiving thiouracil alone (stock diet) and those receiving thiouracil and AAF (corn meal diet) with and without additional thermen. These adenomatous lesions occurred more frequently and were more extensive in animals receiving AAF in addition to thiouracil than in those receiving the goitrogen alone, but were otherwise similar in appearance. (Figs. 3 and 4).

In no case was it felt possible to make a diagnosis of malignancy on the basis of purely morphological or cytological characteristics. Lesions were classified as malignant on the basis of infiltration between

TABLE III: INFLUENCE OF METHIONINE ON THE INCIDENCE OF CANCER OF THE LIVER

	AAF	AAF Testost. prop.	AAF Methionine	AAF Testost. prop. Methionine
Number of rats	33	20	16	14
Incidence, cancer of liver, %	73	80	87.5	79
Weight,* Cancerous livers	12.3	15.1	8.8	9.5
Weight,* Non-cancerous livers	6.7	8.5	9.7	7.6

* gm. per 100 gm. body weight

TABLE IV: INCIDENCE OF ADENOMA AND CARCINOMA OF THE THYROID

	1 Thiouracil stock diet	2 AAF Thiouracil	3 AAF Thiouracil Testost. prop.	4 AAF Thiouracil Estradiol diprop.	Summary 2 to 4: All animals Throuracil,AAF	AAF Thiouracil Thyroxine
Number of rats	20	28	13	6	47	32
Duration of exper., days	245–884	154–316	164–249	149–174		147–279
Adenoma of thyroid	11 (55%)	28 (100%)	11 (84.6%)	6 (100%)	44 (93.6%)	25 (78.1%)
Carcinoma of thyroid	1 (5%)	5 (17.3%)	2 (15.3%)	—	7 (14.8%)	—

apy. Inasmuch as no sex difference was observed, the data are not separated on this basis.

The thyroid was increased in size and weight in all animals receiving thiouracil; the degree of enlargement varied with the duration of treatment with this agent and was not significantly affected by simultaneous administration of AAF or sex hormones. The isthmus was usually involved in the enlargement. The glands were firm, sometimes lobulated and pale brown or gray in color.

Microscopic examination uniformly revealed the characteristic hyperplasia induced by thiouracil and similar goitrogenic drugs. Papillary proliferation appeared to be less marked after very long periods of therapy than in earlier stages, the process resembling the microfollicular rather than the macrofollicular and papillary hyperplasia of Graves' disease (14). Some of the follicles, especially in the subcapsular region, contained pink-staining colloid. Occasional groups of acini were separated by connective tissue septa containing fibrocytes and lymphocytes.

Irregular nodular areas of two types were observed within the hyperplastic thyroid tissue. One consisted of islets or solid cords of cells. The other consisted of dilated acini, lined by low columnar or cuboidal cells and filled with red-staining colloid, with occasional finger-like projections into the lumuscle fibers, invasion of blood vessels and metastasis to the lungs. (Figs. 5 and 6).

Administration of sex hormones had no appreciable influence upon the incidence of tumors of the thyroid. Estradiol was not well tolerated by rats receiving AAF and thiouracil, only 6 surviving 149 to 174 days and none surviving beyond this period. In the group of 47 rats receiving AAF and thiouracil, the incidence of adenoma was 93.6 per cent and of carcinoma 14.8 per cent as compared with 55 and 5 per cent, respectively, in the 20 animals receiving thiouracil alone. The one instance of thyroid malignancy in the latter group occurred after 884 days of treatment, whereas the 7 instances of malignancy in the AAF-treated group were observed after 232 to 312 days.

DISCUSSION

The data presented indicate (a) that thiouracil, administered in the drinking water, protects the liver of rats against the induction of cystic and neoplastic lesions by 2-acetaminofluorene and (b) that this protection extends also to the aggravating influence of testosterone upon these processes (4, 15). Similar, but less complete protection was observed against *p*-dimethylaminoazobenzene. Attempts to elucidate the mechanism of action of

thiouracil in this connection were inconclusive. Three possibilities suggest themselves immediately:

1. Inasmuch as both the AAF and the thiouracil were ingested, the latter, perhaps as a reducing agent, might act upon the former in the gastrointestinal tract, converting it to an inactive compound.

2. The phenomenon may be dependent upon the state of hypothyroidism induced by thiouracil.

3. Thiouracil may act upon some enzyme mechanism in the liver cell in such a manner as to prevent the carcinogenic influence of AAF. The possibility of decreased intake of food and, therefore, of carcinogen, by animals receiving thiouracil was eliminated by measurement of food intake and weight gain, which did not differ significantly from the control (AAF) group.

The occurrence of tumors of the bladder, ductus acusticus, breast, uterus and thyroid in the absence of hepatic involvement in animals receiving thiouracil and also the restoration of carcinogenicity by injecting thyroxine, militate against the possibility of inactivation of the carcinogen in the bowel. This possibility is being explored further by administering AAF and thiouracil by different routes.

The role of hypothyroidism cannot be evaluated properly on the basis of the data obtained. Thyroidectomized rats did not tolerate the diet and carcinogen long enough to yield pertinent information. The fact that thyroxine, administered simultaneously, counteracted the protective influence of thiouracil is difficult to interpret. These agents exert no direct effect upon one another (1, 10). Although the quantity of thyroxine injected was adequate to have suppressed thyroid hyperplasia in rats receiving thiouracil (5, 11, 13), no such suppression was effected in the group receiving thiouracil and AAF. One cannot, therefore, attribute the restoration of hepatic carcinogenicity of AAF by thyroxine to prevention of thiouracil-induced hypothyroidism without additional information regarding the BMR in these animals. It is possible that the carcinogenic mechanism in the hepatic cell is responsive to quantities of thyroxine that are inadequate to maintain normal thyroid structure. On the other hand, it is also possible that thiouracil may exert a direct effect upon certain metabolic processes in the liver cell independently of its thyroid-depressing action (9). The failure of added methionine to combat the carcinogenic effect of AAF is of interest in this connection in view of the suggestion by György and Goldblatt (6) that the protective influence of thiouracil against dietary cirrhosis may be due to decreased requirement for (*e.g.*, conservation of) methionine incident to the induced state of hypothyroidism.

The data presented indicate that, in rats receiving AAF, the hepatic "cocarcinogenic" effect of testosterone is accompanied by a decrease in its androgenicity, and that this phenomenon is reversed by simultaneous administration of thiouracil. This suggests that, in the presence of AAF, the metabolism of testosterone, perhaps in the liver, may be perverted so as to produce a metabolite that is cocarcinogenic and only slightly androgenic; when the liver is protected, by thiouracil, against the carcinogenic action of AAF, testosterone takes its normal metabolic pathway. The possibility was considered that the effect of thiouracil in increasing the androgenicity of testosterone in AAF-treated rats may be due to increased sensitivity of the target organs in the hypothyroid state, such as has been reported for estrogen (8). However, there is no evidence that this holds true for androgens, and, in unpublished experiments in our laboratories, administration of thiouracil to rats receiving testosterone (no AAF) had no perceptible effect on the androgenicity of the latter.

Our findings confirm the observation (2, 3, 12) that administration of goitrogenic drugs (allyl thiourea, thiouracil, etc.) leads to the formation of adenomas and, if continued for long periods, carcinoma of the thyroid in rats. Simultaneous ad-

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Testis of a rat which received AAF and testosterone propionate for 248 days, showing well preserved spermatogenesis. Hematoxylin and eosin stain. Mag. × 200.

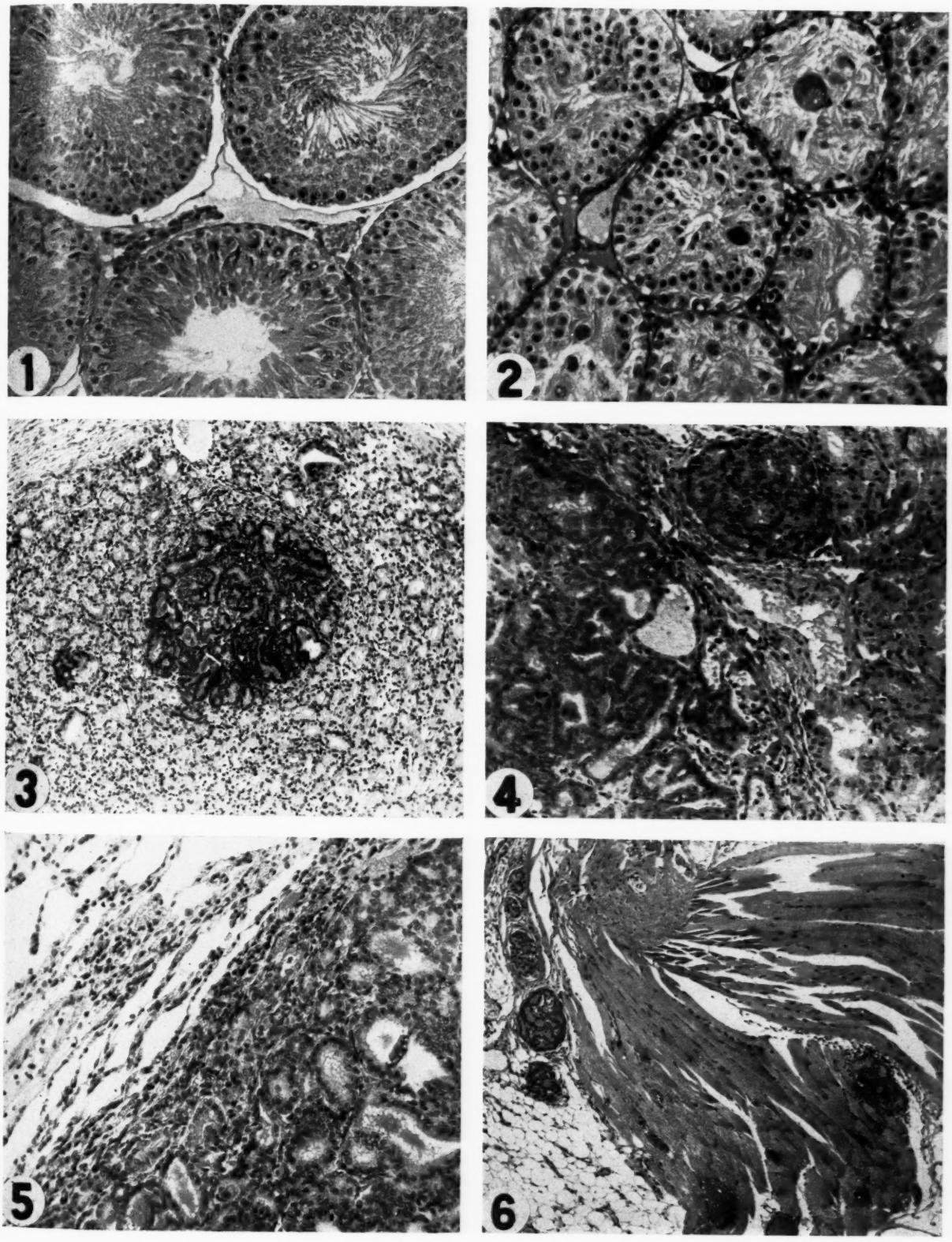
FIG. 2.—Testis of a rat which received AAF, testosterone propionate and thiouracil for 164 days, showing degenerative changes in seminiferous tubules and absence of mature sperm. Hematoxylin and eosin stain. Mag. × 200.

FIG. 3.—Thyroid of a female rat treated with AAF and thiouracil for 312 days. Adenoma in the hyperplastic thyroid (microfollicular type). Hematoxylin and eosin stain. Mag. × 80.

FIG. 4.—Thyroid of a female rat treated with AAF, thiouracil and estradiol diprop. for 179 days, showing adenoma with hyperchromatic nuclei and irregular arrangement of acini. Hematoxylin and eosin stain. Mag. × 150.

FIG. 5.—Lung of female rat treated with AAF and thiouracil for 304 days, showing metastasis from thyroid cancer, resembling "benign metastasizing adenoma". Hematoxylin and eosin stain. Mag. × 180.

FIG. 6.—Thyroid cancer invading blood vessels and skeletal muscle fibers. Male rat treated with AAF and thiouracil for 293 days. Hematoxylin and eosin stain. Mag. × 115.



Figs. 1-6

ministration of AAF accelerates the development and increases the incidence of these lesions.

SUMMARY

1. Simultaneous administration of thiouracil protected the liver of rats against the carcinogenic action of 2-acetaminofluorene (AAF). This protection was exerted also against the hepatic cocarcinogenic action of testosterone. The incidence of liver carcinoma induced by *p*-dimethylaminoazobenzene was lowered in females by administration of thiouracil.

2. Possible mechanisms whereby this effect may be produced are discussed. The possibility that hypothyroidism plays an important role in this connection cannot be eliminated. However, a direct action of thiouracil upon liver cells, independent of the induced state of hypothyroidism, cannot be excluded from consideration.

3. Quantities of thyroxine adequate to prevent thyroid hyperplasia in rats receiving thiouracil alone did not inhibit hyperplasia in those receiving thiouracil and AAF.

4. Administration of methionine did not decrease the incidence of carcinoma of the liver induced by AAF.

5. Testosterone exerted only a mild androgenic effect (prostatic weight increase and inhibition of spermatogenesis) in rats receiving AAF. In those receiving also thiouracil, testosterone evoked the expected androgenic response. The hypothesis is suggested that, in the presence of AAF, the metabolism of testosterone is perverted so as to form a hepatic cocarcinogenic metabolite which is only weakly androgenic. When the action of AAF upon the liver is prevented by thiouracil, testosterone takes its normal metabolic pathway.

6. Thyroid adenomas and carcinoma developed in rats receiving thiouracil alone for protracted periods. Simultaneous administration of AAF hastened the development and increased the incidence of these lesions.

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A Further Report on Yolk Sac Cultivation of Tumor Tissue

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About five years ago a technic was described by means of which rat and mouse tumors could be grown in the yolk sacs of embryonated eggs (5, 7). At that time, it was pointed out that by this method relatively large masses of pure cultures of rat and mouse tumor tissue were readily obtainable. Other laboratories have used the technic successfully (1, 2, 8).

Since the publication of the initial reports, this manner of growing tumor tissue has been used continuously in this laboratory. Both rat and mouse tumors have been carried over a period of years by egg to egg inoculation.

The present report is made with the object, first, of describing the yolk sac technic as modified by experience with several hundred thousand inoculations and, secondly, to report the discovery of a C3H mammary carcinoma which grows with unusual facility by this method of cultivation.

I. Yolk Sac Method of Growing Tumor Tissue

The technic as presently carried out in this laboratory can best be described under three headings as follows: (a) harvesting and preparation of tumor tissue, (b) preparation of the egg for inoculation, and (c) inoculation and incubation of the injected eggs.

(a) *Harvesting and preparation of tumor tissue.*

(i) *Egg to egg inoculation.*—Tumors are usually harvested from the egg on the 17th or 18th day of incubation. The eggs are dipped in 70 per cent ethanol and then opened carefully into Petri dishes (size 100 × 15 mm.). Each Petri dish receives the contents of one egg and is immediately covered.

Ten or 12 eggs are prepared in this manner before proceeding further. The tumor is recovered by means of small scissors and forceps. The yolk sac is opened and the tumor tissue which appears essentially as it does in the normal host, is freed from the surrounding yolk and placed in a staining dish or Petri dish. Large pieces of tissue are cut up to facilitate transfer to the homogenizing syringe. Only about 4 gms. of tumor are placed in each dish, since this is the maximum quantity used in each homogenizing syringe. A little saline poured over the tissue washes off some of the adherent yolk and tends to prevent clogging of the syringe.

The homogenizing syringes may be of 5 or 10 cc. capacity. The 5 cc. size is preferred here for egg-grown tumor tissue. The syringe is fitted with stainless steel screens cut to fit the barrel. Fine screens are 30 mesh, 0.0114 gauge. Coarse screens are 20 mesh, 0.016 gauge. "Coarse screen" syringes consist of 2 coarse screens in between 2 fine screens placed at the bottom of the barrel. "Fine screen" syringes consist of 1 fine screen placed at the bottom of the barrel. Where the tissue is less easily homogenized, a "bead" syringe is used, consisting of a layer of glass beads in between 2 coarse screens. About 4 cc. of tumor tissue is introduced into a 5 cc. "coarse screen" syringe. The plunger is reinserted and the contents are forced out into a 5 cc. "fine screen" syringe which has a 2 inch 20 gauge needle on it. The second syringe receiving the homogenized tissue serves both to measure the amount of tumor tissue and to screen out any oversized particles which may have escaped the first syringe. The material is now ready for mixing with the suspending fluid.

(ii) *Mouse to egg inoculation.*—The animals are etherized, decapitated, and immersed in iodized ethanol. The tumor tissue is removed and placed in the staining dishes. Care is taken to eliminate pieces of connective tissue which are likely to make the homogenization more difficult. The balance of the technic is similar in all respects to that given for egg to egg inoculation.

The suspending medium for the tumor tissue consists of a fluid made up of ½ egg white and ½ 0.85 per cent saline. By means of a 5 cc. syringe with a 2 inch 18 gauge needle the egg white is drawn up directly from the egg. A small hole is made in the large end of a fertile egg which has been incubated the same length of time as the eggs which are to be inoculated. With the egg in a vertical position the needle is inserted for its full 2 inches and 2 cc. (for 1:4 dilution) of the egg white is drawn up into the syringe. The needle of the syringe is now dipped into the saline and an

equal amount is taken up. The contents of the syringe are then expelled into an 8 cc. test tube (10 × 1.5 cm.) which is fitted with a sterile cork in preference to a cotton plug (because of the lint contamination). As many tubes are prepared in this manner as will be necessary for the inoculation of the particular group of eggs.

Each tube receives 1 cc. (1:4 dilution) of the homogenized tumor tissue. In depositing the tissue into the tube, the needle is inserted below the surface of the liquid before pressing the plunger. Mixture of the suspending liquid and tumor tissue is accomplished, when ready to inject the eggs, by carefully drawing up material into the 1 cc. syringe and then expelling it back into the tube. Only a moderate amount of mixing is necessary. Too finely dispersed mixtures may result in smaller tumors.

(b) *Preparation of the eggs for inoculation.*—Fertile eggs are incubated 3 to 4 days in preparation for the injection of the tumor tissue suspension. The amount of incubation is calculated on the basis of the embryo development. At various seasons of the year, there is a difference in the stage of embryo growth at the time the eggs are placed in the incubator. In the winter, it requires about 4 days of incubation at 37.2° C. (99° F.) for the eggs to reach the required stage of development. In the summer (in Austin) about 3½ days are sufficient. The diameter of the area vasculosa as seen through the shell serves as an index of the stage of embryo growth. It has been found that at the time of inoculation the diameter of the vascular area should be between 3.5 and 4.5 cms. for the best results. Eggs much under the 3.5 cms. stage tend to a lower survival rate and those much above 4.5 cms. will tend to produce smaller tumors. It is the practice in this laboratory to outline the area vasculosa and the air sac space with a wax pencil at the time the eggs are candled in preparation for inoculation.

With the point of one blade of a pair of scissors a small hole is tapped into the shell of each egg in the area over the air space. The shell is perforated, but the underlying membranes are not. The eggs are kept with the same side uppermost so that the embryo will be above the needle at the time of injection.

(c) *Inoculation and incubation of the injected eggs.*—Using a 1 cc. syringe and 1¼ inch 20 gauge needle, ½ cc. of the mixture of tumor and saline-egg white suspension is injected into the yolk sac. A needle with bevel up, penetrating the egg through the air sac for a distance of 1 to 1¼ inches parallel to the long axis, enters the yolk sac. The plunger is pressed with moderate force expelling the contents into the egg.

The yolk at the stage of development attained at the time of inoculation is quite thin and watery and the force of the injection carries some of the tumor tissue to the inner wall of the yolk sac. It is here that the vascularization with chick blood vessels occurs (3).

The opening through the air sac is closed with cellulose tape. At the same time identifying marks and other data are written on the egg with a wax pencil.

During the preparation and inoculation process, the eggs may be kept at room temperature. After injection the eggs have been incubated at 37.2° C. (99° F.) and given the usual care accorded to hatching eggs. Dead eggs are removed daily and opened to obtain data on infection and survival.

Tumor-bearing eggs are sensitive to temperature variation especially to rises in temperature above 37.2° C. Data obtained on the comparative survival of tumor-bearing eggs for different incubators appear to bear out this statement. Where temperature control was less dependable the egg mortality increased markedly. In experiments carried out to test this factor figures were obtained on the egg survival of two incubators. The only pertinent difference between the two machines was the fact that incubator A tended to vary plus or minus 1° C. with an occasional deviation of as much as 1.5° C., while incubator B showed a variation of about 0.2° C. and was quite dependable at that temperature. In a series of 9 experiments including more than 400 eggs for each machine, incubator A had a survival rate of 34.1 per cent and incubator B, 48.6 per cent in this respect.

Sterilization and asepsis.—Little need be said about the necessity for rigid asepsis in this work. Surgical asepsis is not adequate. It is the practice here to wrap instruments and glassware in jewelers' paper and bake in an oven for 4 hours at 220° C. (425° F.). Petri dishes, used in large numbers, are baked in special containers. Saline solutions and wrapped test tube corks are autoclaved 30 minutes at 15 lbs. pressure.

Small laboratories are desirable where the egg work can be done undisturbed. Sterile lamps are unnecessary if the room is kept reasonably free of dust and if the air currents from open windows or doors or the moving about of workers is avoided. Surgical masks are worn by the technician and assistants.

Tumors used in egg work.—Numerous varieties of mouse tumor have been grown successfully in eggs by the yolk sac method of cultivation. A

mammary carcinoma originating spontaneously in the dba strain was the first tumor to be successfully cultivated in this manner. It has now been carried by egg to egg transplant for 115 generations or about 4 years. During that time there has been no observable change in the histology of the tumor. There has been a decrease in egg survival from about 60 per cent to a little above 40 per cent of the number injected (Table I). Also another change in the chick embryo-tumor relationship has appeared. It is now not uncommon for the embryo from the egg carrying the dba mammary carcinoma to show malformations such as blister-like structures at various places on the surface of the body. These "blisters" consist of a transparent membrane filled with a clear fluid. Such structures were not observed during the early egg to egg series.

TABLE I: DATA ON TUMOR SIZE AND SURVIVAL OF EGG-GROWN DBA MAMMARY CARCINOMA

Transplant generation in eggs	No. of eggs injected	Egg survival, 17 day %	Av. tumor wt., gms.
109	41	65.0	1.1±0.1
109	75	64.0	0.9±0.1
109	36	52.8	1.3±0.2
109	72	50.0	1.1±0.1
112	41	56.1	1.0±0
112	28	40.7	0.9±0.1
110	46	54.3	1.0±0
110	75	29.0	0.6±0.4
110	39	24.3	1.0±0
111	41	35.9	1.1±0.1
113	43	53.5	0.7±0.3
113	61	66.7	0.7±0.3
113	49	57.4	1.0±0
111	42	51.3	1.3±0.3
111	64	54.0	1.3±0.3
Average	50	50.3	1.0

Many other mouse tumors, both sarcomas and carcinomas, have been grown in eggs. Most of them have not shown as good a growth as that manifested by the original mammary carcinoma. Considerable variation has been noted in the growth response of mouse tumors to the egg environment. It has been found that mammary carcinomas originating in the same strain and with about the same growth rate in mice may differ to the extent that one will show little or no growth while another shows good growth in the egg. It has been noted too, that after a few passages a particular tumor may grow more readily in the egg.

Very little work has been done here with rat tumors. The Walker 256 has been carried continuously by egg to egg inoculation for 64 transplant-generations. This tumor grows more readily and consistently in eggs. It has been found much easier and more economical to carry the Walker tumor in eggs than in the host animal. The egg culture is always available for animal transplants. The rat tumors produced by the implantation of dba mouse tumor tissue into the anterior chambers of the eyes of the rats (6) were cultivated in eggs. These tumors, so readily transplantable in the rat showed vigorous growth also in eggs.

II. EGG CULTURE OF A C3H MAMMARY CARCINOMA

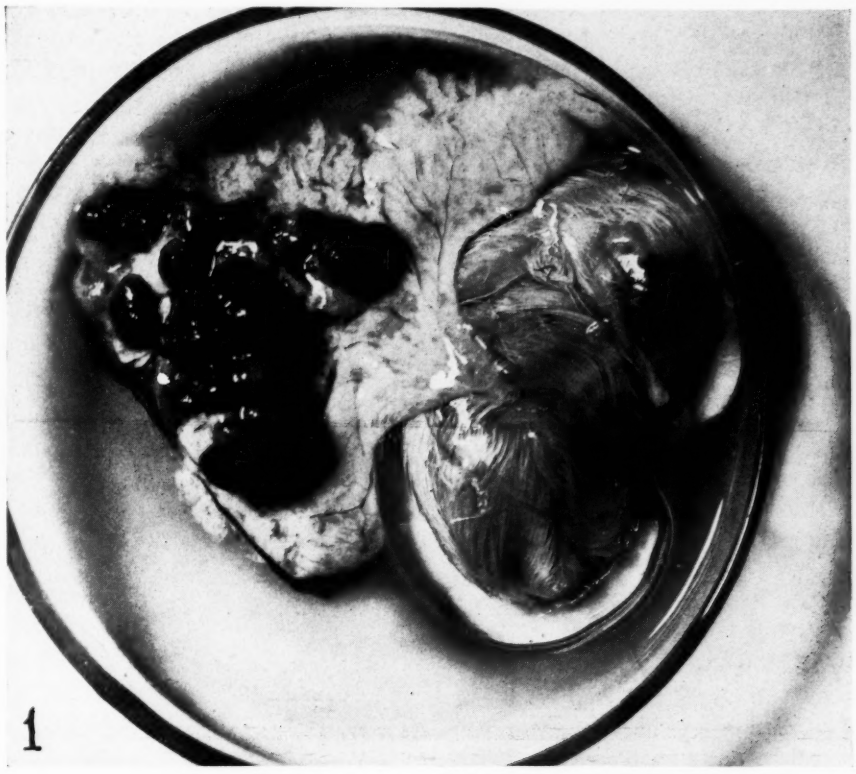
Recently a mammary tumor that occurred spontaneously in a C3H female has been found to grow with unusual vigor when inoculated into embryonated eggs. Five and 6 gm. tumors are common 13 to 14 days after egg implantation. Table II reveals the tumor size and egg survival for each of 15 individual experiments. The gross appearance and histology of this tumor as it appears when egg-grown are shown in Figs. 1 and 2. When grown by transplants in the host mouse its gross and microscopic morphology appears to be identical with the egg-cultivated tissue.

TABLE II: DATA ON TUMOR SIZE AND SURVIVAL OF EGG-GROWN C3H MAMMARY CARCINOMA

Transplant generation in eggs	No. of eggs injected	Egg survival, 17 day %	Av. tumor wt., gms.
6	30	40.0	3.6±1.0
6	28	46.4	3.7±1.5
6	18	38.9	3.5±1.3
10	30	48.1	4.1±1.1
7	19	68.4	3.4±1.3
7	24	34.5	2.4±1.1
7	27	50.0	3.5±0.9
11	18	50.0	2.5±1.8
8	30	48.3	2.0±1.3
8	50	42.9	1.7±1.2
12	72	34.7	2.5±1.3
12	29	39.3	1.9±1.5
13	31	56.7	2.1±0.8
13	60	35.0	2.4±1.3
10	19	42.1	4.2±1.0
Average	32	45.0	2.9

The growth rate of the new tumor is higher in the eggs than in the host C3H mouse. A group of 25 mice showed an average tumor size of 2.4 gms. 17 days after implantation, while the egg-grown tumors averaged 2.9 gms. 13 to 14 days after egg inoculation (Table IV).

The C3H mammary tumor has been carried continuously by egg to egg inoculation for 16 generations. It is interesting to note how it differs in its effect on the chick embryo in comparison with the dba mammary carcinoma which has been carried in eggs for 114 transplant-generations. Table II discloses that the average tumor size of the C3H tumor was nearly 3 times that of the dba. At the



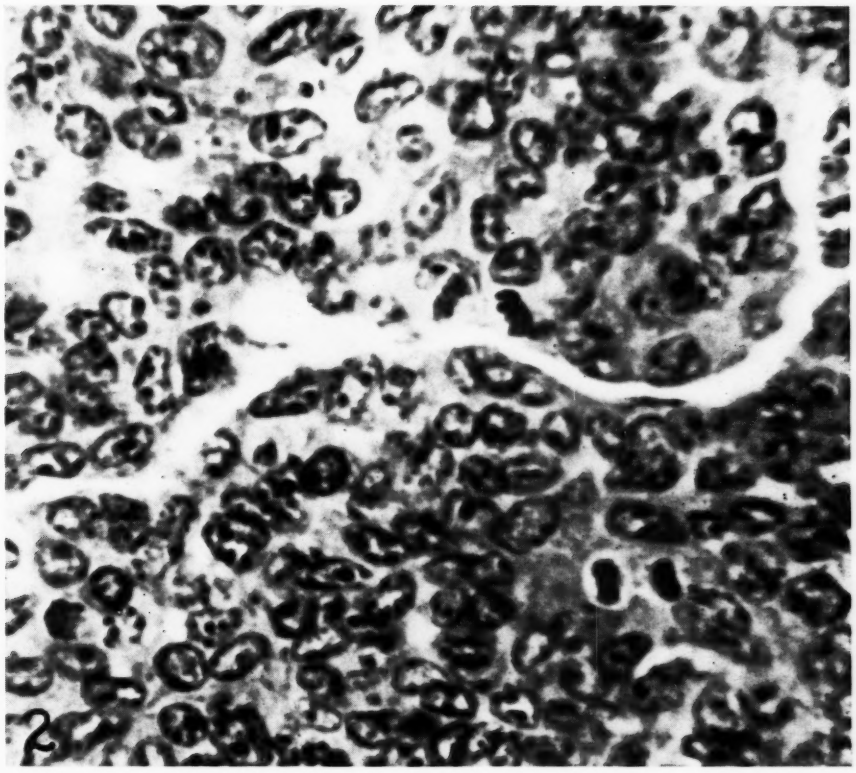


FIG 1.—C3H mammary carcinoma as it appears in the yolk sac.

FIG. 2.—Histological section of the egg-cultivated C3H mammary carcinoma shown in Fig. 1. Mag. × 1000.

same time the chick embryo was appreciably larger in association with the C3H mouse tumor as compared with the dba mouse tumor (Table III). It has been shown previously (4) that the chick embryo size is inversely related to the size of the egg-grown tumor. It appears from the present data that some factor in addition to tumor size was concerned with the inhibition of the embryo growth.

TABLE III: EFFECT OF DBA AND C3H MOUSE TUMOR ON CHICK EMBRYO SIZE

Name	No. of eggs	Av. tumor wt., gms.	Av. embryo wt., gms.	Ratio experimental embryo to control, %
Uninjected control	100	....	21.7±1.4	100.0
dba	100	0.9±0.2	12.7±2.0	58.5
C3H	100	2.4±0.5	15.3±2.1	70.5

TABLE IV: COMPARATIVE TUMOR SIZE AND EMBRYO SURVIVAL ASSOCIATED WITH EGG-GROWN DBA AND C3H MAMMARY CARCINOMA

Name	No. of experiments	Total no. injected	Survival, %	Av. tumor wt., gms.
C3H	15	485	45.0	2.9±1.2
dba	15	753	50.3	1.0±0.2

The C3H tumor has not affected the liver of the chick embryo to the same extent as the old dba tumor, and the blister-like structures have not been observed as yet.

DISCUSSION

The yolk sac method of propagating tumor tissue in embryonated eggs has now been thoroughly tested over a period of 4 years. This technic is simple enough that after a week or so of supervision new technicians can do the work without difficulty. The routine associated with the inoculation of 8 doz. eggs requires about 2 hours for one person. This includes the time it takes to harvest and prepare the tumor tissue and to prepare the eggs for inoculation. It does not include the sterilization of the required apparatus.

Tumor tissue grown in eggs is made up of a pure culture of cancer cells. Non-malignant tissue of the mouse or rat has not been maintained in the egg beyond the first transplant. All the non-cancerous tissue associated with the egg-grown tumor is of chick origin.

It would seem that for many types of research the isolation of malignant from non-malignant cells would be desirable. It can be done by tissue culture, but the egg technic produces masses of tumor tissue comparable to the quantities obtained by transplants in the host animal. This method of obtaining tumor tissue would be especially useful in immunological studies.

The question has naturally arisen as to the possibility of cultivating human tumor tissue in a continuous egg to egg series. It has been considered that the relatively slower growth rate of most human cancer tissue would preclude its cultivation in eggs. However, the growth rate of a tumor in the egg may be much greater than it is in the host animal. This has been shown for the C3H mammary carcinoma considered in this paper. Further, some human tumors have a relatively high growth rate. It would be especially important in this work to get the tumor tissue in the eggs as soon as possible after removal from the patient. This appears to be so on the basis of experience with rat and mouse tumor tissue.

The project of finding a human tumor suitable for continuous egg cultivation might take some time, but a successful outcome would justify considerable effort.

SUMMARY

1. A report is made of the yolk sac technic of growing tumor tissue as modified by experience with hundreds of thousands of egg inoculations.

2. Details are also given of a C3H mammary carcinoma which grows with unusual vigor in the egg environment. In a series of 15 experiments totaling 485 eggs, this tumor averaged 2.9 gms. 13 to 14 days after implantation in the egg.

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A Tumor in a Fresh-Water Mussel*

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INTRODUCTION

The description of tumors in invertebrates is rare. Smith in 1934 (4) described a mesenchymal tumor in the oyster (*Ostrea virginica.*) This tumor had arisen from the pericardium, was nodular or polypoid and consisted of large oval mesenchymal cells with an outer layer of simple columnar ciliated epithelium. A similar tumor was found in an oyster in 1887 by Ryder (2), and another tumor was observed in 1890 by Williams (6) in the fresh water mussel *Anodonta cygnea.* The tumor from *Anodonta cygnea* was described as a polypoid adenofibroma from the pallium.

Tumors have also been seen in ants by Brun (1), and in Drosophila by Stark (5). Scharrer (3) induced tumors experimentally by cutting the recurrent nerve in an insect. It is the purpose of this paper to describe a connective tissue tumor with an epithelial covering in the mussel *Anodonta implicata.*

OBSERVATIONS

This specimen was found among mussels imported for zoology classes from the Marine Biological Supply House (Woods Hole, Mass.). The shell of the mussel measured 5.8 by 13.1 cm. and the visceral mass 3.3 by 6.0 cm. The tumor arose from the inner labial palp of the right side and measured 0.8 cm. in diameter and 2.0 cm. in length. Its surface was nodular or polypoid, the nodules being of unequal size (Fig. 1).

Region adjacent to the tumor.—The inner surface of the palp was covered by folded simple columnar epithelium bearing long cilia. The outer surface of the palp was smooth with no folds and its epithelium was for the most part stratified squamous, but in certain regions columnar. The columnar cells of the inner surface, which were continuous with the tumor epithelium, were long and narrow, with spindle-shaped nuclei. No granules were seen in the cytoplasm of these cells. Scattered among the epithelial elements were goblet cells with large vacuoles.

The connective tissue included irregularly arranged fibers of variable thickness. The connective tissue cell nuclei were well stained but the cytoplasm was scant. Among the fibers were numerous masses of connective tissue cells filled with yellow pigment. Some of these cells were nucleated, others were not. Spaces containing blood were observed among the fibers. The white blood corpuscles frequently had eccentrically placed nuclei and some had granular cytoplasm.

The tumor.—The columnar epithelium from the folded inner surface of the palp continued over the nodular surface of the tumor (Fig. 2). The columnar cells were smaller and less regular than those of the inner surface of the palp. Numerous goblet cells with a large vacuole were noted and occasionally secretory granules were found (Fig. 3).

The pigmented connective tissue cells were entirely absent from the tumor. The connective tissue cells were very abundant near the periphery of the nodules but scanty in the center. Mitotic figures were very few. The connective tissue cells resembled those in the connective tissue of the palp, but were somewhat larger and their cytoplasm was more granular. The connective tissue fibers were like those of the normal region; however there was an abundance of blood sinuses as compared with the normal area. These vascular sinuses contained white blood corpuscles with eccentric nuclei, some of them possessed cytoplasmic granules and others did not. White corpuscles were found invading the connective tissue cells in some abundance.

SUMMARY

A connective tissue tumor, lined by simple columnar ciliated epithelium, arising from the palp of the mussel *Anodonta implicata,* is described.

ACKNOWLEDGMENT

The author wishes to thank Professor William M. Shanklin, Director of the Histology Department, for supervising this work.

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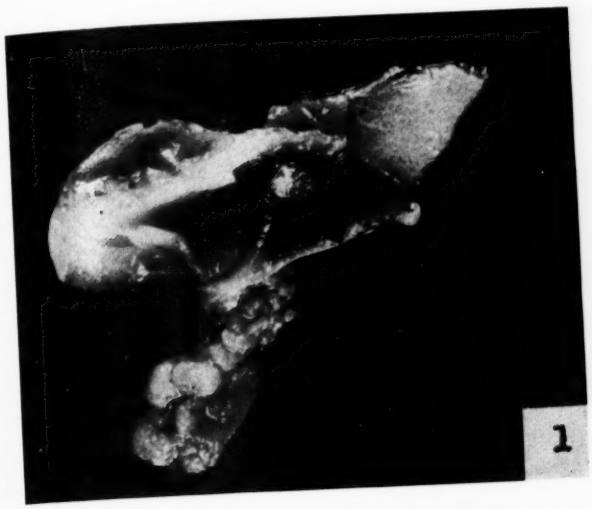

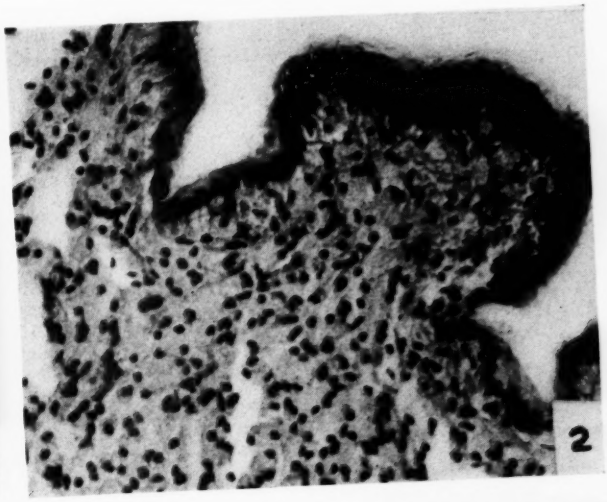

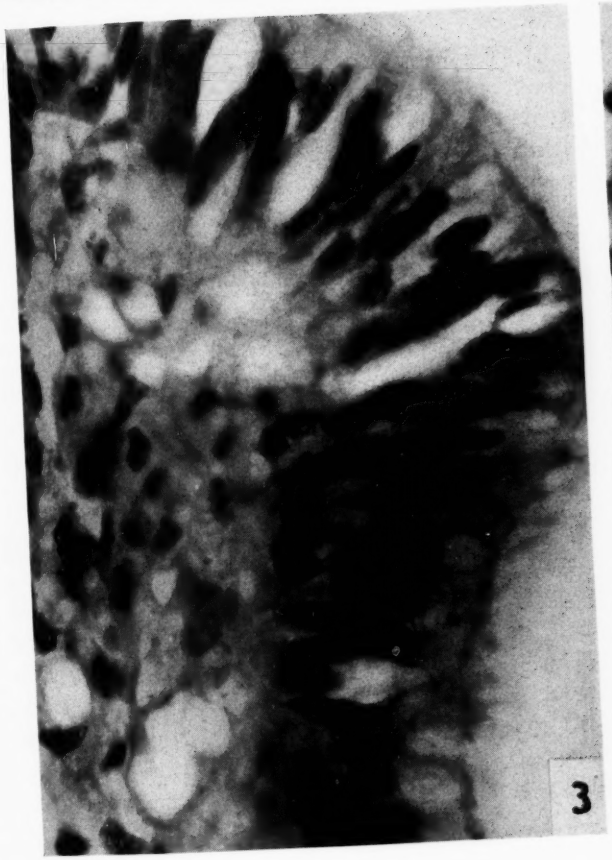

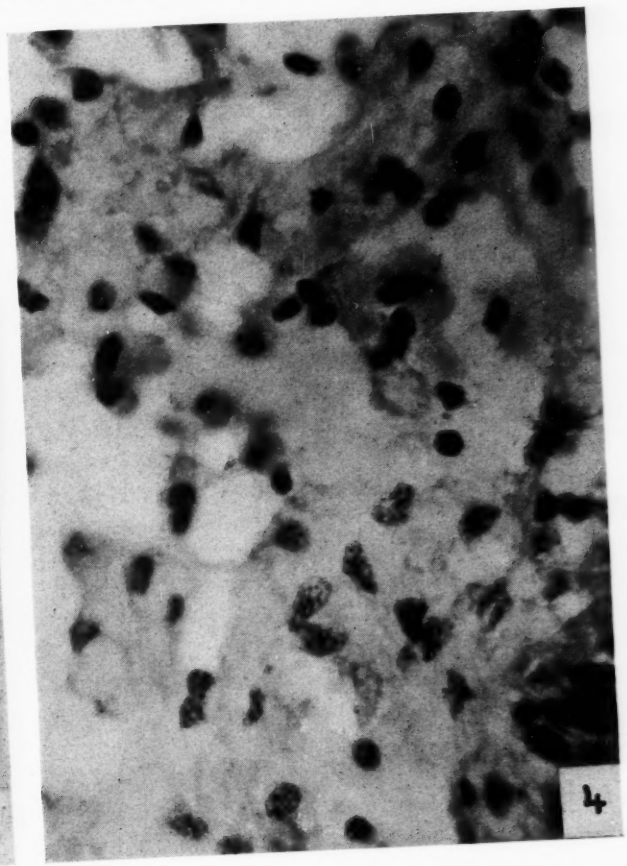

FIG. 1.—Connective tissue tumor with part of the mouth region, slightly enlarged, from *Anodonta implicata*.

FIG. 2.—Section of the tumor from *Anodonta implicata*. Hematoxylin-eosin stain. Mag. × 150.

FIG. 3.—Peripheral region of tumor from *Anodonta implicata* showing epithelial lining. Hematoxylin-eosin stain. Mag. × 700.

FIG. 4.—Central region of tumor showing numerous blood sinuses and corpuscles among the connective tissue cells. Hematoxylin-eosin stain. Mag. × 700.

Heterologous Transplantation of Cancer of Childhood*

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The high degree of anaplasia and the early metastatic dissemination observed in many cancers of childhood suggest considerable autonomy of the neoplastic tissue, and readiness to transgress species barriers, as demonstrated by successful transfer into the anterior chamber of the guinea pig's eye.

During the past two years many of the malignant neoplasms submitted to the Department of Pathology of The Children's Hospital, Boston, were inoculated into the anterior chamber of the guinea pig's eye following the technic described by Greene (1). A total of 27 tumors were inoculated into 158 guinea pigs. The number of animals inoculated ranged from 1 to 20 (Table I). Most of these

TABLE I: GUINEA PIGS INOCULATED WITH NEOPLASTIC TISSUE INTO THE ANTERIOR CHAMBER OF THE EYE

Tumor	No. of tumors	No. of animals	Successful	Unsuccessful
Embryoma of kidney	12	74	0	74
Neuroblastoma	5	44	0	44
Fibrosarcoma	2	23	1	22
Osteogenic sarcoma	3	12	0	12
Ewing's tumor	1	3	0	3
Craniopharyngioma	1	3	0	3
Lymphoma	1	2	0	2
Neurofibrosarcoma	1	2	0	2
Rhabdomyosarcoma	1	1	0	1
Total	27	158	1	157

tumors were examples of the two most frequent retroperitoneal neoplasms in childhood, the embryoma of the kidney, and the neuroblastoma. Except in two cases the tissue was derived from surgical material, received sterilely, and within an hour after their removal.

Transplants were made subcutaneously, intraperitoneally, and intracerebrally, also, and into mice, rats, and rabbits. This report deals only with transplants into the anterior chamber of the guinea pig.

Successful heterologous transfers were not obtained with ease. In no instance was a "take" achieved with tissue from the embryomas or neuroblastomas. The only successful transfer was obtained with tissue from a congenital sarcoma, classified at that time as fibrosarcoma; in this case the growth began after a long incubation period (157 days), and in only one animal out of 18.

This neoplasm arose in the soft tissue of the calf of a newborn female infant (Fig. 1, 2 and 3) with a high cancer incidence in the maternal family. The tumor was poorly differentiated, (Fig. 4), metastasized early and widely, and led to death on the 98th day of life. The successful transfer was done on the 33rd day of life, following biopsy of the tumor; transfer of metastatic tissue, at autopsy, was not successful.

The tumor tissue, at the time of the successful inoculation, was soft, pale, and friable. The inoculation was done between 1 and 3 hours after surgical removal. It had been kept at room temperature, and suspended in sterile physiological saline solution most of this time.

A total of 18 animals were inoculated. In 12 of these the inoculum had been practically absorbed within 3 weeks, leaving only faint scars. One died of broncho-pneumonia after one week. No tumor tissue was found microscopically in the eye of this animal. One animal showed absorption after 8 weeks. In one animal an attempt at vascularization occurred after 5 weeks. Autopsy 2 weeks later showed granulation tissue only. A similar attempt was seen in another animal, after 12 weeks; this attempt was followed by absorption.

One animal, the twelfth of this series, was inoculated more than 2 hours after surgical removal. The inoculum remained stationary for 3 weeks, being located in the center of the anterior chamber, without apparent connection to the iris. During the following 5 weeks a faint gray, slowly growing halo was noted around the inoculum. Vascularization took place after 8 weeks, with the development of a small pink mass filling about one-fourth of the anterior chamber. This regressed, however, and at the end of 3 months only a pale gray scar remained. During the fourth month there was again some vascular growth, accompanied by what appeared to be a hemorrhage into the anterior chamber. Three weeks later another small pink mass was noted which grew steadily during the following 2 weeks to occupy a space measuring 0.6 × 0.6 cm. The animal was then sacrificed, 157 days after inoculation. Microscopic sections showed tissue

* Supported by Grant No. 250 of the National Cancer Institute, U. S. Public Health Service, Bethesda 14, Maryland.

more differentiated than the original tumor, and resembling a fibrosarcoma (Fig. 5).

Five animals were inoculated with the tissue obtained from the eye. In 3 of these the inoculum was absorbed within 2 weeks. One has remained stationary for 4 months. In another animal there was subtotal absorption during the first month. Vascularization and growth occurred during the first half of the second month, but again regression took place during the second half of this month. Ten weeks after inoculation vascularization again occurred, with increase in the size of the inoculum. The animal was sacrificed, 79 days after inoculation. The sections showed a fibrosarcoma, more cellular than after the first transfer, but still more differentiated than the original tumor (Fig. 6). Four more animals were inoculated with the growth obtained, but total absorption had occurred in all animals within 150 days.

DISCUSSION

It is reasonable to assume that the reported experiment represents a successful heterologous transplant of human neoplastic tissue. The morphological picture, the long incubation period, and the gross observations during this time make unlikely an inflammatory process of the iris of the host.

There is at present no explanation for the failure of the other inoculations.

SUMMARY

A successful heterologous transplant of a congenital human fibrosarcoma into the anterior chamber of a guinea pig's eye is reported. Heterologous transplants of 26 other human cancers of childhood, mostly embryomas and neuroblastomas, were unsuccessful.

NOTE: Since this paper was submitted, a successful heterologous transplant of an embryoma of the kidney was achieved in one out of 6 animals. There was a latent period of about 2 months. The microscopical structure was identical with that of an embryoma. Second generation transplantation into 2 animals was unsuccessful.

ACKNOWLEDGMENT

I wish to thank Mr. Robert Chaney, Mr. William Walker, and Mr. John Carabitses for their valuable assistance.

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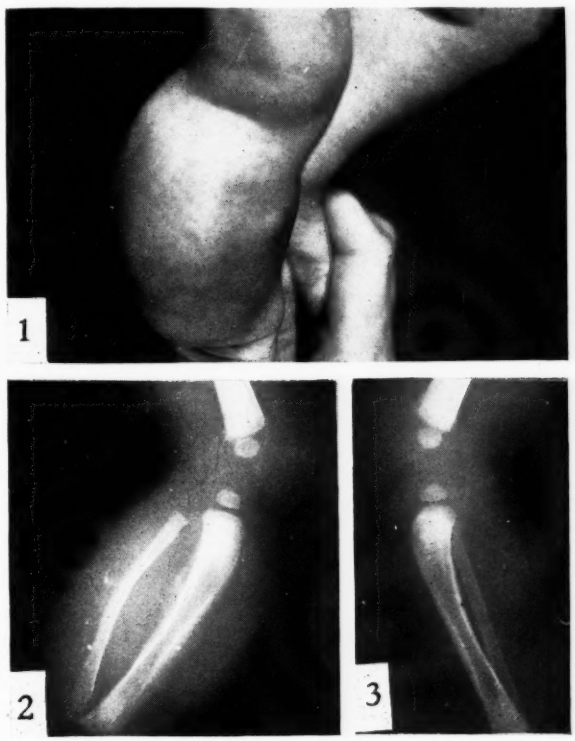
DESCRIPTION OF FIGURES 1 TO 3

FIG. 1.—Congenital fibrosarcoma of 33 days old infant, from which grafts were taken.

FIG. 2.—Roentgenogram of right calf showing involvement of soft tissue.

FIG. 3.—Roentgenogram of normal left calf of female infant.



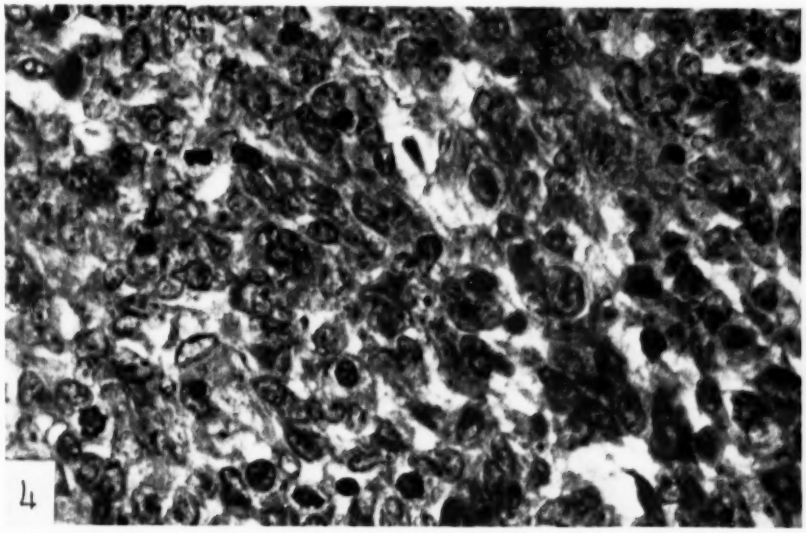
Figs. 1-3

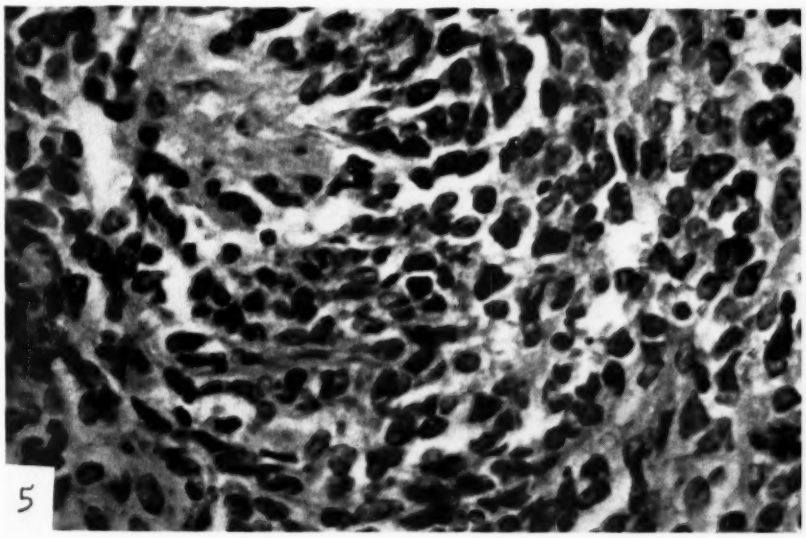
DESCRIPTION OF FIGURES 4 TO 6

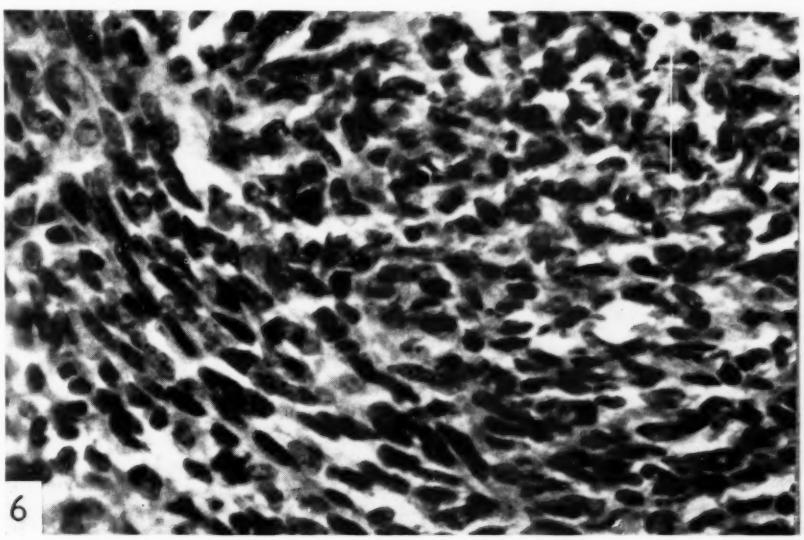
FIG. 4.—Section of biopsy from congenital fibrosarcoma, (S–46–907).

FIG. 5.—First generation transplant in guinea pig's eye after 157 days.

FIG. 6.—Second generation transplant after 79 days.







Figs. 4-6

Hepatic Riboflavin and Tumor Formation in Rats Fed Azo Dyes in Various Diets*

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It is well recognized that rats fed *p*-dimethylaminoazobenzene (DAB) develop tumors at rates that depend upon the composition of the basal diet. Dietary factors reported to increase the rate of tumor formation under certain circumstances include biotin (2, 4), pyridoxine (16), cystine (18), and the rice bran concentrate "Vitab" (12, 13, 16). The dietary factors most effective in retarding tumor formation include riboflavin (8, 12, 16) and hydrogenated coconut oil (13, 14). The evidence for these effects has been summarized in a recent review (17). Later experiments, however, suggest that riboflavin may be more important in the development of hepatic tumors than most of the other dietary factors that have been studied. When *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*-Me-DAB) was fed as the carcinogen, the rate at which tumors developed was not increased by the feeding of the rice bran concentrate, nor did hydrogenated coconut oil exert any considerable retarding effect (6). Riboflavin, however, retarded the development of tumors due to the *m*-methyl dye, though not as much as when DAB was the carcinogen. Further evidence for a peculiar role of riboflavin in the formation of hepatic tumors is the observation that certain of the azo dyes markedly lower the hepatic concentration of this vitamin (7, 9) while chemically similar non-carcinogenic dyes do not (7). Indeed, there appears to be a rough correlation between the carcinogenic potencies of the various azo dyes and their effects upon the concentration of riboflavin in the liver in relatively short-term experiments (7). The question, therefore, arose whether diets known to alter the rate of hepatic tumor formation might not exert their effects primarily by modifying the ability of the liver to store riboflavin.

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METHODS

The experiments were of two general types. The first involved series in which hepatic tumors were produced in rats by feeding them diets similar to those used in previous experiments but designed to indicate the relative effectiveness of riboflavin, hydrogenated coconut oil, and the rice bran concentrate "Vitab" in altering the rate of formation of tumors due to *p*-dimethylaminoazobenzene. The diets contained 5 per cent of either corn oil or hydrogenated coconut oil (HCNO), in which enough *p*-dimethylaminoazobenzene was dissolved to furnish a concentration of 0.06 per cent of the dye in the ration. The B vitamins were added either as the synthetic mixture (16, 17) used routinely in previous experiments, or as the rice bran concentrate, "Vitab." Comparisons between these two sources of the B vitamins were made at two concentrations of riboflavin in the diet, 0.3 and 2.0 mgm./kgm. When the diet contained corn oil, the dye was fed for 4½ months, at which time the livers of a few animals in each group were examined by laparotomy. Thereafter, the corresponding basal diets free from the dye were fed for two more months, when the animals were killed and the livers removed for examination. The animals fed hydrogenated coconut oil received the dye for 5 months. The composition of the various diets is indicated in Table I.

The second type of experiment was primarily analytical. Rats of various ages were fed either *p*-dimethylaminoazobenzene or *m'*-methyl-*p*-dimethylaminoazobenzene in diets selected for their known ability to modify the rate of tumor formation. The diets (Table I) included our standard synthetic ration (16, 17) containing corn oil (diet 1), a similar diet in which the corn oil was replaced by hydrogenated coconut oil (13, 14) (diet 3), a diet containing the rice bran concentrate "Vitab" (diet 2), and another containing additional amounts of riboflavin (16) (diet 1). In other diets the level of casein was raised from 12 to 24 per cent, or higher amounts of riboflavin or of the rice bran

concentrate were incorporated (Tables III and IV). Such additions were made at the expense of the glucose. After 3 to 6 weeks the animals were killed by decapitation and the livers removed, weighed rapidly, and homogenized in a Waring blendor with 10 volumes of 0.1 *N* H_2SO_4. The mixture was autoclaved for 15 minutes at 15 pounds pressure, and the riboflavin determined fluorometrically according to the method of Conner and Straub (3), as modified by Andrews (1).

EXPERIMENTAL

Relative effectiveness of riboflavin, hydrogenated coconut oil, and the rice bran concentrate "Vitab" on tumor formation.—In line with previous observations in this laboratory, tumors due to *p*-dimethylaminoazobenzene developed more rapidly on a diet containing the rice bran concentrate, "Vitab," than when the standard synthetic diet was fed. These two diets are known to differ in their content of riboflavin. In a subsequent series 0.06 per cent *p*-dimethylaminoazobenzene in corn oil was fed in either the "Vitab" diet, or the synthetic diet, modified to contain equivalent amounts of riboflavin. Comparisons were made at 2 levels of the vitamin: 2.0 mgm. per kgm. and 0.3 mgm. per kgm. Tumors developed most rapidly at the lower level of riboflavin regardless of the source of the other B-vitamins. When the diet contained 2 mgm. of riboflavin per kgm., the incidence of tumors at 6½ months was 30 per cent on both the synthetic and the "Vitab" diets (Table II, groups 5 and 6). When the diets contained 0.3 mgm. of riboflavin per kgm. the incidence of tumors on the two diets was approximately 87 per cent at this time (Table II, groups 7 and 8). In other words, the incidence of tumors was increased by a reduction in the concentration of riboflavin in the ration whether synthetic B vitamins were fed or whether the diet contained the rice bran concentrate.

TABLE I: COMPOSITION OF DIETS

	gm./kgm.			
	Corn oil synthetic	Corn oil vitab*	HCNO† synthetic	HCNO† vitab*
	1	2	3	4
Cerelose‡	790	770	790	770
Casein (purified)	120	120	120	120
Salts	40	40	40	40
Corn oil§	50	50		
Hydrogenated coconut oil			50	50
Rice bran concentrate		20		20
Thiamine	.003		.003	
Pyridoxine	.0025		.0025	
Choline	.030		.030	
Calcium pantothenate	.007		.007	
Riboflavin	.0003–.02	0–.002	.0003–.002	0–.002

*Vitab = Rice bran concentrate, National Oil Products Company, Harrison, New Jersey.
†HCNO = Hydrogenated coconut oil, Lever Brothers, Cambridge, Massachusetts.
‡Cerelose = Pure glucose monohydrate.
§ Corn oil = Mazola, Corn Products Refining Company.

TABLE II: RELATIVE IMPORTANCE OF RIBOFLAVIN AND OTHER NUTRIENTS IN MODIFYING THE DEVELOPMENT OF TUMORS DUE TO *p*-DIMETHYLAMINOAZOBENZENE

	Diet fed								Tumor incidence		
Group	Vitamin source		Riboflavin conc. mgm./kgm.	Time dye was fed months	Av. initial wt. gm.	Av. wt. at end of dye feeding gm.	Av. food consumption gm./rat/day	Survival at end of dye feeding	at end of dye feeding	2 months later	Cirrhosis 2 months after end of dye feeding
1	Corn oil	Vitab	0.7	4	225	196	10.0	12/15	0/12	7/12	5
2	Corn oil	Synthetic	2.0	4	224	221	10.8	14/15	1/14	5/13	0
3*	Corn oil	Vitab	0.7	3	100	130	9.3	10/10	1/10	4/8	3
4*	Corn oil	Synthetic	2.0	3	97	164	9.8	10/10	1/10	5/10	5
5	Corn oil	Synthetic	2.0	4.5	160	194	10.0	10/10		3/10	5
6	Corn oil	Vitab	2.0	4.5	158	250	9.7	10/10		3/10	3
7	Corn oil	Synthetic	0.3	4.5	159	157	7.5	7/10	4/10	6/7	6
8	Corn oil	Vitab	0.3	4.5	161	162	8.7	9/9		7/8	7
9	HCNO	Synthetic	2.0	5	172	202	11.6	15/15		0/15	0
10	HCNO	Vitab	2.0	5	171	212	11.4	14/15		0/14	0
11	HCNO	Synthetic	0.3	5	165	142	8.7	14/15		7/12	mild
12	HCNO	Vitab	0.3	5	174	159	8.5	13/15		7/13	mild

*All groups except 3 and 4 were fed 0.06 per cent *p*-dimethylaminoazobenzene; groups 3 and 4 received 0.032 per cent *m'*-methyl-*p*-dimethylaminoazobenzene.
Corn oil—Mazola, Corn Products Refining Company.
Vitab—Rice bran concentrate, National Oil Products Company, Harrison, New Jersey.
HCNO—Hydrogenated coconut oil, Lever Brothers, Cambridge, Massachusetts.

This conclusion was verified in another series in which the fat of the diet was hydrogenated coconut oil (Table II, groups 9 to 12). Hepatic tumors due to *p*-dimethylaminoazobenzene are known to develop more slowly in the presence of hydrogenated coconut oil than of corn oil (13, 14). Hence in the present series the dye was fed for 5 months, and an additional 2 months permitted on the basal diets free from dye before the final examination for tumors was made. With hydrogenated coconut oil no tumors developed on either the synthetic diet or the "Vitab" diet when 2.0 mgm. of riboflavin were present per kgm. of diet (Table II, groups 9 and 10) although in the presence of corn oil an incidence of 30 per cent had been noted in the previous series in which the carcinogen was fed for a shorter period of time (Table II, groups 5 and 6). Thus, the protective effect of hydrogenated coconut oil against tumors due to *p*-dimethylaminoazobenzene (13, 14) was again confirmed. At the lower level of riboflavin intake, however, tumors developed in the presence of hydrogenated coconut oil on both the "Vitab" and synthetic diets. The incidences were nearly identical, 58 and 54 per cent respectively at 7 months (Table II, groups 11 to 12). This similarity in response would appear to indicate that the rice bran concentrate does not possess any peculiar factors that stimulate hepatic tumor formation other than the relatively low riboflavin content of the preparation.

Incidentally, at the lower level of riboflavin intake, hydrogenated coconut oil also retarded tumor development: 56 per cent of the animals on the coconut oil diet developed tumors after ingesting the dye for 5 months (Table II, groups 11 and 12) as compared to an incidence of 87 per cent when the dye was fed for 4½ months in comparable diets containing corn oil (Table II, groups 7 and 8).

Effect of various diets on the storage of riboflavin in the liver.—In general those diets on which rats are known to resist tumor formation tended to maintain relatively normal concentrations of riboflavin in the liver; those on which tumor formation is rapid tended to depress the concentration of hepatic riboflavin. The results were not always consistent, variations within groups were sometimes wide, and in individual rats in which the liver was enlarged abnormally under the influence of the azo dye, the concentration of hepatic riboflavin was sometimes depressed even though the total amount of the organ may have been quite high. A summary of the results is as follows: The livers of rats fed the rice bran concentrate, "Vitab," and 0.06 or 0.09 per cent of *p*-dimethylaminoazobenzene averaged 14.0 μgm. of riboflavin per gm. (11.7-16.8) and 74 μgm. per total liver (37-126) (Table III, groups 17, 26, 31, 37) while livers from corresponding rats fed the synthetic diet averaged 16.2 μgm. of riboflavin per gm. (13.7-18.3) and 97 μgm. per total liver (37-126) (Table III, groups 15, 24, 29, 35). In corresponding pairs of groups either the concentration or the total amount of riboflavin was higher on the synthetic diet than on that containing the rice bran concentrate (Table

TABLE III: EFFECT OF CERTAIN DIETS ON THE RIBOFLAVIN CONTENT OF LIVERS OF RATS FED *p*-DIMETHYLAMINOAZOBENZENE (DAB)

Group	Diet and dye fed	Casein level gm./kgm.	Feeding period wks.	μgm. B_2 per gm. /diet	No. of animals	Initial wt. gm.	Growth increment gm./wk.	Average daily food intake gm.	Average liver wt' gm.	Liver riboflavin μgm./gm.	Range	Liver riboflavin Total μgm.
13	Corn oil Syn. 0.06% DAB	120	3	2.0	5	108	0	8.0	5.3	14.4	13.5–15.5	75
14	HCNO Syn. 0.06% DAB	120	3	2.0	5	109	+5	9.0	5.9	17.1	15.7–19.6	101
15	Corn oil Syn. 0.09% DAB	120	3	2.0	3	200	−7	9.2	7.6	15.8	14.3–16.8	121
16	HCNO Syn. 0.09% DAB	120	3	2.0	3	185	0	10.6	7.3	18.3	16.7–20.0	134
17	Corn oil Vitab 0.09% DAB	120	3	0.7	3	200	−3	10.0	6.2	15.3	15.0–15.8	95
18	Corn oil Vitab (4%) 0.09% DAB	120	3	0.85	3	200	−5	9.4	7.4	14.4	13.2–15.8	107
19	Corn oil Syn. 0.09% DAB	120	3	10.0	3	200	−5	11.0	7.3	19.2	17.8–20.8	139
20	Corn oil Syn. 0.06% DAB	120	3	2.0	3	143	−1	11.0	7.0	16.2	13.8–17.5	113
21	HCNO Syn. 0.06% DAB	120	3	2.0	3	147	−2	9.5	6.5	18.2	17.3–18.9	118
22	Corn oil Vitab 0.06% DAB	120	3	2.0	3	148	−4	9.0	6.2	17.4	17.3–17.5	108
23	Corn oil Syn. 0.06% DAB	240	3	2.0	3	143	−11	10.0	6.1	20.2	19.0–21.0	123
24	Corn oil Syn. 0.09% DAB	120	6	2.0	3	215	−6	9.5	6.5	15.0	13.7–17.0	98
25	HCNO Syn. 0.09% DAB	120	6	2.0	3	228	−4	9.9	9.2	13.6	13.0–14.2	125
26	Corn oil Vitab 0.09% DAB	120	6	0.7	3	212	−10	9.0	5.3	13.3	12.5–14.6	70
27	Corn oil Syn. 0.09% DAB	120	6	10.0	3	225	−3	11.4	8.0	20.1	17.0–22.7	161
28	Corn oil Syn. No dye	120	6	2.0	6	190	+11	13.0	9.0	17.2	15.0–19.0	155
29	Corn oil Syn. 0.09% DAB	120	6	2.0	3	186	−3	8.8	5.5	17.0	16.6–17.9	95
30	HCNO Syn. 0.09% DAB	120	6	2.0	3	205	−1.5	11.6	7.5	17.5	16.8–18.2	130
31	Corn oil Vitab 0.09% DAB	120	6	0.7	3	188	−3	10.3	6.3	15.2	13.5–16.8	96
32	Corn oil Vitab (4%) 0.09% DAB	120	6	0.85	3	170	+2	9.4	6.3	15.5	14.6–17.0	97
33	Corn oil Syn. 0.09% DAB	120	6	10.0	3	195	−5	9.0	5.8	26.0	22.0–30.0	150
34	Corn oil Syn. No dye	120	6	2.0	5	125	+12.5	14.1	6.5	19.0	19.0–21.0	124
35	Corn oil Syn. 0.09% DAB	240	6	2.0	3	57	+5	6.3	4.4	16.7	15.2–18.3	75
36	HCNO Syn. 0.09% DAB	240	6	2.0	3	56	+7	6.9	5.0	19.0	15.0–25.0	94
37	Corn oil Vitab 0.09% DAB	240	6	0.2	3	56	+2	5.4	3.1	12.3	11.7–12.9	37
38	Corn oil Syn. 0.09% DAB	240	6	20.0	3	55	+9	8.4	6.0	23.5	23.4–23.5	141

Corn oil—Mazola, Corn Products Refining Company; HCNO—Hydrogenated coconut oil; Vitab—Rice bran concentrate.

III, group 15 vs. 17, 24 vs. 26, 29 vs. 31). The differences are regarded as suggestive, rather than as completely significant. When the riboflavin content of the diet was increased, the storage of the vitamin in the liver increased significantly (Table III, groups 19, 27, 33 and 38 versus groups 15, 24, 33 and 35), even though fairly high amounts of *p*-dimethylaminoazobenzene were present in the diet. This is the counterpart to the well-known effect of riboflavin in retarding the development of tumors due to this dye.

In the presence of *m'*-methyl-*p*-dimethylaminoazobenzene the storage of riboflavin in the liver was

When hydrogenated coconut oil was substituted for corn oil in diets containing *p*-dimethylaminoazobenzene, an increase in hepatic riboflavin usually resulted. This was true with both weanling and adult rats, whether the dye was fed as 0.06 per cent or 0.09 per cent of the ration, or whether the feeding time was 3 or 6 weeks (Table III, groups 15 and 20 versus 16 and 21). Thus, for groups 15 and 20 the concentration of riboflavin per gram of liver averaged 16.0 μgm./gm. (15.8-16.2) on corn oil as contrasted to 18.3 μgm./gm. for groups 16 and 21 on hydrogenated coconut oil. Maximum differences due to these fats were observed in two

TABLE IV: EFFECT OF CERTAIN DIETS ON THE RIBOFLAVIN CONTENT OF LIVERS OF RATS FED *m'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE (*m'*-ME-DAB)

Group	Diet and dye fed	Casein level gm./kgm.	Feeding period wks.	μgm. B_2 added per gm. diet	No. of animals*	Initial wt. gm.	Growth increment gm./wk.	Average daily food intake gm.	Average liver wt. gm.	Liver riboflavin μgm./gm.	Range	Total μgm.
39	Corn oil Syn. 0.096% *m'*-Me-DAB	120	3	2.0	2/4	140	−9	6.5	4.7	12.8	11.8–13.9	60
40	HCNO Syn. 0.096% *m'*-Me-DAB	120	3	2.0	4	140	−7.7	6.6	5.0	11.7	10.0–14.0	59
41	Corn oil Vitab 0.096% *m'*-Me-DAB	120	3	0.7	4	142	−7.7	6.3	4.5	12.3	10.7–14.0	55
42	Corn oil Vitab—5% 0.096% *m'*-Me-DAB	120	3	0.5	4	123	−7.2	5.6	3.7	11.4	9.8–14.0	42
43	Corn oil Syn. 0.096% *m'*-Me-DAB	120	3	10.0	5	142	−7.6	6.9	5.1	14.9	12.0–17.9	76
44	Corn oil Syn. control No dye	120	3	2.0	5	123	+12.5	14.1	6.4	19.3	17.6–21.0	124
45	Corn oil Syn. Restricted No dye	120	3	2.0	5	142	−10.0	4.0	3.4	29.0	21.0–34.0	99
46	Corn oil Vitab No dye	120	3	0.7	5	132	+10.0	13.9	5.8	18.8	17.2–21.0	110
47	Corn oil Syn. 0.096% *m'*-Me-DAB	120	6	2.0	3/10	250	−9	7.2	6.9	13.6	12.8–14.7	94
48	HCNO Syn. 0.096% *m'*-Me-DAB	120	6	2.0	5/10	275	−14	7.2	6.7	13.5	12.0–15.5	91
49	Corn oil Vitab 0.096% *m'*-Me-DAB	120	6	0.7	2/10	276	−15	7.0	7.0	11.6	10.3–12.8	82
50	Corn oil Syn. 0.096% *m'*-Me-DAB	120	6	10.0	5/10	268	−9	8.2	8.3	14.8	13.5–16.4	123
51	Corn oil Syn. 0.064% *m'*-Me-DAB	240	6	2.0	3/10	53	+5	6.0	5.7	10.7	10.5–11.0	61
52	HCNO Syn. 0.064% *m'*-Me-DAB	240	6	2.0	3/10	53	+6	5.8	5.6	11.8	11.0–13.5	66
53	Corn oil Vitab 0.064% *m'*-Me-DAB	240	6	0.7	3/10	55	+3	5.5	4.3	11.7	9.8–15.0	50
54	Corn oil Syn. 0.064% *m'*-Me-DAB	240	6	10.0	3/5	57	+7	6.7	9.3	13.2	12.0–15.0	123
55	Corn oil Vitab 0.064% *m'*-Me-DAB	240	6	2.2	3/5	47	+7	5.5	6.0	11.3	9.5–13.0	68
56	Corn oil Syn. control No dye	240	6	2.0	3	61	+29	14.0	7.7	18.0	15.3–21.5	139
57	Corn oil Syn. control No dye	240	6	10.0	5	50	+20	13.8	6.5	24.8	21.0–27.0	160

Corn oil—Mazola, Corn Products Refining Company; HCNO—Hydrogenated coconut oil; Vitab—Rice bran concentrate.
* Fractions indicate number surviving the experimental period. Analytical results apply only to those that survived the full period.

low whether the corn oil synthetic diet or the diet containing the rice bran concentrate was fed (Table IV, groups 39 versus 41, 51 versus 53). Variations within groups were usually wider than variations between groups although the average riboflavin storage was slightly lower on the "Vitab" diets. Previous experiments have indicated that the incidence of tumors is essentially the same on these two types of diets when the carcinogen is *m'*-methyl-*p*-dimethylaminoazobenzene (6). The addition of comparatively high levels of riboflavin to the synthetic diets containing the *m'*-methyl dye resulted in increased hepatic riboflavin storage, *e.g.*, 12.8 μgm./gm. versus 14.9 μgm./gm., and 60 μgm. versus 76 μgm. per total liver (Table IV, groups 39 and 43). Thus the improvement in riboflavin retention was not so marked with *m'*-methyl-*p*-dimethylaminoazobenzene as with *p*-dimethylaminoazobenzene (Table III), and this difference between dyes parallels the degrees to which their carcinogenic activities can be counteracted with dietary riboflavin (6, 16).

other series in which 0.09 per cent of the azo dyes was fed. The total amounts of riboflavin per liver averaged 96 μgm. on corn oil as contrasted to 127 μgm. on hydrogenated coconut oil (Table III, groups 24 and 29 versus 25 and 30).

In contrast to this increase in liver riboflavin observed in the presence of *p*-dimethylaminoazobenzene, hydrogenated coconut oil failed completely to maintain riboflavin storage when the carcinogen was *m'*-methyl-*p*-dimethylaminoazobenzene (Table IV, groups 39 versus 40, 47 versus 48). This observation is the counterpart to previous data (6) indicating that hydrogenated coconut oil does not diminish the rate of tumor formation when the *m'*-methyl dye is the carcinogen.

DISCUSSION

It has been suggested that several of the dietary combinations known to modify tumor formation may do so through a common mechanism, and present results indicate that this is at least partly true, since the "Vitab" diet, which accelerates tumor for-

mation due to *p*-dimethylaminoazobenzene, tended to decrease the concentration of hepatic riboflavin while diets containing hydrogenated coconut oil, which retard tumor formation, permit a greater retention of the vitamin than the control synthetic diet containing corn oil. Alterations in the dietary intake of riboflavin, as expected, alter the amount in the liver and change the rate of tumor formation correspondingly. Thus the original experiments of Kensler and associates (9) indicating a loss of hepatic riboflavin in the presence of azo dyes, are confirmed and extended, and it now appears that there is an inverse relationship between the rate of tumor formation on any particular dietary combination and the concentration of hepatic riboflavin retained on that diet. Experiments with the more active carcinogen, *m'*-methyl-*p*-dimethylaminoazobenzene also support this conclusion. Hepatic riboflavin was particularly low when this potent carcinogen was fed ([7], and Table IV versus Table III) and the storage of the vitamin was not modified by the rice bran diet, nor by hydrogenated coconut oil. This parallels previous results indicating that tumor formation due to the *m'*-methyl dye is relatively insensitive to these dietary factors (6). However, additional riboflavin in the diet decreased the rate of tumor formation due to *m'*-methyl-*p*-dimethylaminoazobenzene (6), while present data (Table IV) indicate that riboflavin retention is also increased somewhat when more of the vitamin is fed.

The inverse relationship between hepatic riboflavin and tumor formation raises the question whether the loss of hepatic riboflavin, at least locally, may be a necessary prerequisite for tumor formation. One might postulate a competitive inhibition between the azo dyes and riboflavin (or between their derivatives) for a critical spot on an enzyme molecule with the excess dye crowding out the riboflavin and exerting adverse effects. Conversely, excess riboflavin might crowd out the carcinogen and prevent it from acting. Substances such as hydrogenated coconut oil would then function by altering the amount of riboflavin available.

Another possibility is that the carcinogenic dyes damage a fundamental liver constituent, *e.g.*, protein, directly, and that the damaged protein is unable to retain riboflavin in normal concentration. In a sense, then, hepatic riboflavin would serve merely as an indicator of changes in other substances in the liver. Dietary factors such as hydrogenated coconut oil, low fat (10), or high levels of corn oil (10), might then exert their effects upon the primary carcinogenic reaction without themselves reacting with riboflavin. It would then also be possible, as observed by Kensler and his associates (8), for extremely high supplements of riboflavin (5 mgm./rat/day) to fail to prevent tumor formation provided the basal diet were otherwise inadequate. However, some other explanation would be needed for the fact that riboflavin alone retards tumor formation when added to a diet moderately low in protein ([16] and Table II). A connection between hepatic riboflavin and resistance to tumor formation has also been established in extensive experiments by Miller, Miller, Kline, and Rusch (11, 15).

SUMMARY

1. Hepatic tumors due to *p*-dimethylaminoazobenzene (DAB) developed more rapidly on a diet containing the rice bran concentrate, "Vitab," than on a standard synthetic diet. The two diets contained 0.7 and 2.0 mgm. of riboflavin per kgm., respectively. When the riboflavin content of the two diets was equalized, tumor formation was also equal. Reduction in riboflavin intake on both diets accelerated tumor formation.

2. When DAB was fed to rats in the "Vitab" diet low in riboflavin, the concentration of this vitamin in the liver tended to decrease more rapidly than when the control synthetic diet was fed. The addition of riboflavin to either diet increased hepatic retention of the vitamin and decreased tumor incidence. Thus, retention of riboflavin in the liver paralleled resistance to tumor formation with this carcinogen.

3. When *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*-Me-DAB) was fed in the "Vitab" and synthetic diets respectively, the amounts of hepatic riboflavin retained were equally low. These diets do not affect the rate at which tumors develop when *m'*-Me-DAB is fed.

4. Livers of rats fed DAB in hydrogenated coconut oil contained more riboflavin than when corn oil was fed. The coconut oil retards tumor formation by this dye. However, when *m'*-Me-DAB was fed in the two oils, the amounts of hepatic riboflavin were equally low; tumor formation due to *m'*-Me-DAB is essentially the same on both oils.

5. The addition of 10 mgm. of riboflavin per kgm. of diet resulted in a marked increase in hepatic riboflavin when the carcinogen was DAB, but in only a moderate increase in the presence of *m'*-Me-DAB. Riboflavin is more effective in retarding tumor formation due to the former dye than to the latter.

6. These results, and the observation that the

degree of carcinogenicity of various azo dyes parallels their effectiveness in lowering hepatic riboflavin, suggest the general conclusion that there is an inverse relationship between the rate of tumor development and the level of hepatic riboflavin maintained on any particular dietary regimen.

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Nucleic Acid Content in Intestines of Rats after X-Radiation

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The chemical changes which are produced in tissues by x-radiation, and which later result in morphological changes and clinical effects, are not well understood. Since nucleic acids apparently are fundamental constituents of all cells, changes in the cell content of these compounds offer a means of studying the effects of x-radiation.

This work is a continuation of studies of radiation effects previously reported (1). The destructive action of x-radiation on cancer cells may result from changes produced in nucleic acids. If the mechanism of this action were explained it might be possible to produce similar results affecting only the cancer cells.

The high absorptive capacity of nucleic acids for ultraviolet light of 2,500 A to 2,800 A, with a maximum absorption at 2,600 A, makes possible the determination of the location and amounts of these compounds in histological sections of tissues. The quartz optical system microscope, often called the ultraviolet microscope, in combination with a source of monochromatic light, is a useful instrument for studying the absorptive capacity of tissues for ultraviolet light. This instrument has been used in the present investigations to determine the effect of x-radiation on the amount of nucleic acids in tissues of the rat intestine. Use was made of the Feulgen reaction, ribonucleinase, methyl green-pyronin stain, and extraction of nucleic acids by hot trichloracetic acid to supplement the ultraviolet light absorption technic. The technic of micro-incineration was employed to study the effect of x-radiation on the amount of mineral constituents.

It was found that a comparatively small dose (600 r) of whole-body x-radiation produced decreased amounts of nucleic acids, mineral constituents, and structural proteins in the crypts of Lieberkühn in the intestines of rats.

MATERIALS AND APPARATUS

Male albino rats of medium size, obtained from the Breeding and Laboratory Institute, Brooklyn, N. Y., were used. They were maintained on Fox Food Blox, an Allied Mills product, and allowed water *ad libitum*.

Irradiation was given to the rats in groups of four with an x-ray machine operated at 120 K.V. The rate of irradiation was 13.45 r/min. A copper filter 0.02 inch in thickness was used.

The Zeiss Ultraviolet microscope (2.5 mm., 0.85 N.A. glycerine immersion objective and 7× ocular) with accessory apparatus described elsewhere (3) was employed for photomicrography. The image magnification was 195×.

The microdensitometer described by Enns (4) was modified for use in the determination of film densities. This apparatus (Fig. 1) consists of an RCA 922 photo cell with an S–2 surface and a Victoreen V–32 inverted triode electronic tube housed together in a metal box, and a stable light source (General Electric Company photo enlarging bulb No.211, in a modified Leica enlarger). The housing was constructed so that circular working apertures ranging from 1/16 in. to ⅜ in. could be interposed between the projected image and the photo tube. A slotted film holder was adapted to the enlarger which permitted the film to be moved by hand to any position. Another strip of film which had been exposed and developed to various degrees of density was placed in another slot of the holder; this film was used as "reference" for adjustment of the apparatus for slight current drifts. The electrometer circuit (not shown in the figure) had an overall sensitivity of 10^{-11} amperes on a 10 cm. scale of a non-recording galvanometer.

EXPERIMENTAL PROCEDURE

Rats of approximately equal weights were paired: one was irradiated, the other served as control. It was known that x-radiation caused rats voluntarily to restrict their food intake; therefore, in all experiments more than 24 hours in duration the food consumption of the control animal of each pair was restricted to the amount consumed by the irradiated one. The danger of attributing effects of decreased food intake to x-radiation was minimized in this way.

At the conclusion of experimental periods the rats were decapitated and the tissues that were to be used were removed immediately. Three fixatives were used: (a) 10 per cent neutral formol in absolute ethyl alcohol: this fixative was recommended by Scott (9) for the fixation of tissues for micro-incineration, (b) 10 per cent neutral formol in 0.85

per cent solution of sodium chloride, (c) glacial acetic acid 3 parts to 1 part of absolute ethyl alcohol.

Comparable specimens of tissue from each pair of animals were carried through the fixation and dehydration processes together; they were then embedded in close proximity in the same block of medium. Embedded in this manner the sections on which comparative studies were to be made were always cut at the same stroke of the microtome knife, a procedure that gave reasonable assurance that the two sections were of the same thickness. In this work comparative estimations were always made on such paired sections.

Sections for ultraviolet light absorption studies with the quartz optical system microscope were cut with the microtome set for 3 microns in thickness and were mounted on quartz slides without an adhesive. The mounted sections were dried at 40° C., deparaffinated in xylol and transferred to 95 per cent ethyl alcohol. They were then mounted in glycerol under a quartz cover slip. The mount was sealed with paraffin.

Three to five microscopic fields in each pair of sections on a slide were photomicrographed on the same strip of film at 2,654 A at constant light intensity and equal exposure times. A "background" exposure was made on each film through a clear area of the slide adjacent to the sections.

Densities of the images of the cells on the photographic film varied with the amount of light-absorbing material in the cells. Densities were measured with the microdensitometer. Not less than 150 areas were measured on the negatives obtained of each section of tissue. Density measurements were also made of areas distributed evenly over the "background" film. The data were used to calculate extinction coefficients. It was assumed that the density of the film in the range of exposures used was proportional to the log of the exposure.

Two methods were used to determine whether the amount of ribonucleic acid was changed in sections from x-rayed animals. (a) Ribonucleic acid was removed by treatment of the sections for 8 to 18 hours with crystalline ribonucleinase (0.2 mgm. N/cc. in distilled water). The decrease in values of the extinction coefficients as a result of the enzyme treatment was taken as a measure of the amount of ribonucleic acid removed. Completeness of removal of ribonucleic acid was tested by the methyl green—pyronin stain, according to Brachet (2). (b) Sections were stained with methyl green—pyronin and examined microscopically for differences in intensity of pyronin staining.

Two methods were employed to investigate changes in the amount of desoxyribonucleic acid caused by x-radiation. (a) Differences in extinction coefficients after ribonucleinase treatment and after removal of all nucleic acid by extraction of the sections in 7 per cent trichloracetic acid at 80° C. for 20 minutes. (b) Sections stained by the Feulgen technic as described by Stowell (11) and photomicrographed with tungsten light and a Wratten 2 b filter; densities of the photomicrographic images measured with the densitometer.

Differences in residual protein structural material were ascertained by determination of extinction coefficients of sections after extraction of nucleic acids by hot trichloracetic acid.

Changes in the amount of mineral ash following irradiation were determined by the densitometric measurement of dark-field photomicrographic images of sections microincinerated at 625° C.

The term "nucleic acid" as used in this study includes along with pure nucleic acids any free nucleotides containing the purine or pyrimidine bases, since these are the ultraviolet-light-absorbing substances. Protein absorption at 2,654 A is so small in comparison with nucleic acid that it has been disregarded. The structural framework of the tissue reflects and scatters light; this also affects the photographic film. Absorption alone, as distinguished from the effects of reflection and scattering of light, was measured by the difference between the extinction coefficients taken before and after extraction of the sections with hot trichloracetic acid.

RESULTS

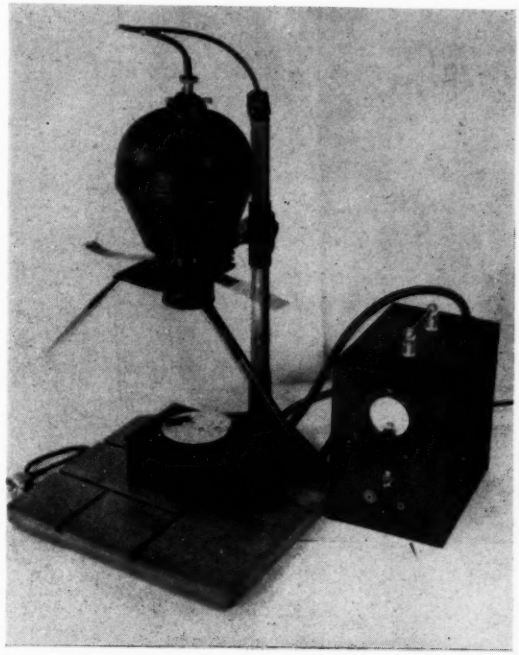
1. *Effects of 24 hours' fasting on the amount of ultraviolet-light-absorbing material in the crypts of Lieberkühn in the duodenum of rats.*—One each of 5 pairs of rats was fasted, but allowed water, for 24 hours. Ultraviolet light absorption by cells in the crypts of Lieberkühn of the duodenum was found to be greater in 4 of the fasted rats than in the corresponding controls, Table I. It was evident

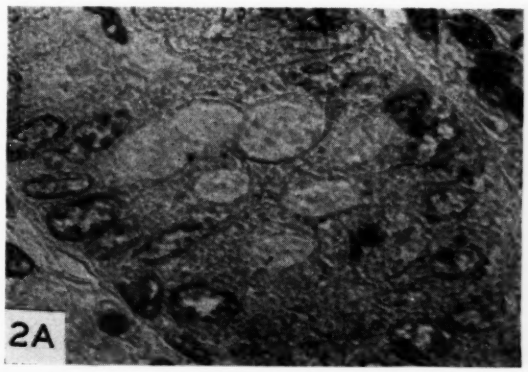
DESCRIPTION OF FIGURES 1 TO 3

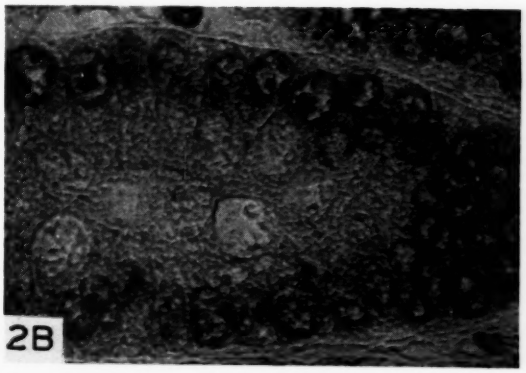
FIG. 1.—Microdensitometer.

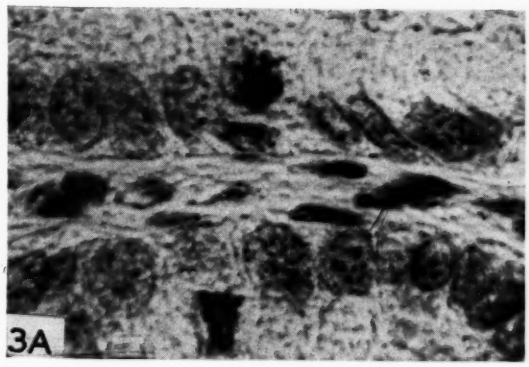
FIG. 2.—Ultraviolet light (2654 A) photomicrographs of crypts of Lieberkühn in ribonucleinase-treated sections from the duodenum of (A) x-rayed (600 r) and of (B) control rats 24 hours after irradiation. Mag. × 875.

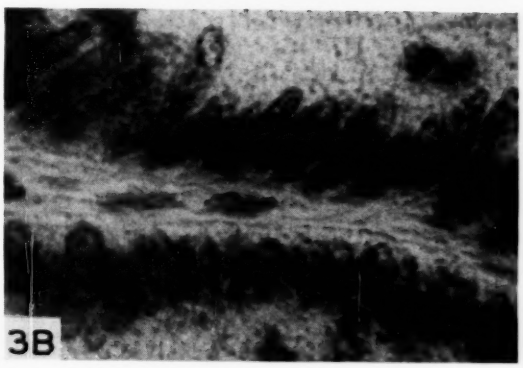
FIG. 3.—Photomicrographs of crypts of Lieberkühn in Feulgen-stained sections taken from duodenum of (A) x-rayed (600 r) and (B) control rats 12 hours after irradiation. Mag. × 830.











Figs. 1-3

TABLE I: COMPARISON OF ULTRAVIOLET LIGHT ABSORPTION BY THE CRYPTS OF LIEBERKÜHN IN THE DUODENUM OF CONTROL RATS AND OF RATS AFTER FASTING FOR 24 HOURS

Rat No.	Extinction coefficients Fasted rats	Fed rats
7633	0.939	0.840
7634	0.721	0.845
7635	0.741	0.705
7636	0.900	0.732
7637	0.937	0.824
Average	0.848	0.789

from the results of this experiment that the voluntary restriction of food intake for 24 hours or less by x-radiated rats would not explain any decreased absorption of ultraviolet light by cells in the crypts of Lieberkühn that might be found in other experiments.

2. *X-radiation effects.*—Four rats were given 600 r x-rays, and 7 days later were given an additional 1,200 r in a preliminary experiment. Sections were taken from the duodenum 20 to 51 hours after completion of irradiation. A great reduction in the amount of ultraviolet-light-absorbing material was found in the crypts of Lieberkühn. Microincinerated sections showed that the mineral constituents also were greatly reduced in amount.

Changes at various levels along the intestinal tract.—Since decreased amounts of ultraviolet-absorbing material and of mineral ash were found in sections from the duodenum of x-irradiated rats in the preliminary experiment, an examination was made of sections taken at different levels of the intestinal tract of rats which had been given 1,000 r in a single dose. The results (Table II), show that in 24 and 48 hours after irradiation, there was a reduction in the quantity of absorbing material in the crypts of Lieberkühn throughout the whole intestinal tract. Absorption by the epithelium of the esophagus also was decreased.

Direct or indirect effects.—In order to determine whether or not reduction in the amount of ultraviolet-light-absorption material in the crypts of Lieberkühn was caused by direct x-radiation of the intestinal tract, rats were given 600 r to the anterior part of the body. Absorption studies were made of tissues taken 24 hours after irradiation. The data presented in Table III show that x-radiation of the head-chest part of the body did not produce the characteristic decrease in ultraviolet-light-absorbing material in the crypts of Lieberkühn found when the whole body was irradiated.

TABLE II: DECREASE IN ULTRAVIOLET LIGHT ABSORPTION IN THE CRYPTS OF LIEBERKÜHN OF THE INTESTINE OF RATS AFTER 1,000 R TOTAL BODY X-RADIATION

Rat No. X-rayed	Rat No. Control	Time after irradiation	Location of section	Extinction coefficients X-rayed rat	Extinction coefficients Control rat	Decrease in absorption, %
7317	7321	24 hrs.	Esophagus (mucosa)	0.459	0.645	28.8
			Duodenum	0.653	1.035	36.9
			6 n. below pylorus	0.725	0.965	24.9
			12 in. below pylorus	0.904	1.139	20.6
			18 in. below pylorus	0.848	1.140	25.6
			30 in. below pylorus	0.949	1.077	11.9
			1 in. below cecum	0.406	0.683	40.6
7318	7319	24 hrs.	Esophagus	0.413	0.758	45.5
			Duodenum	0.991	1.348	26.5
			6 in. below pylorus	0.673	0.932	27.8
			12 in. below pylorus	0.728	1.046	30.4
			18 in. below pylorus	0.722	1.083	33.3
			30 in. below pylorus	0.740	0.799	7.4
			36 in. below pylorus	0.807	1.178	31.5
7320	7323	48 hrs.	Duodenum	0.733	0.869	15.7
			12 in. below pylorus	1.039	1.257	17.3
			18 in. below pylorus	0.872	0.976	10.7
			½ in. below cecum	0.840	0.966	13.0

Duration of decrease in the amount of ultraviolet-light-absorbing material caused by 600 r total-body x-radiation.—A decreased amount of ultraviolet-light-absorbing material was found in the crypts of Lieberkühn in the duodenum in all of 40 rats studied at intervals from 4 hours to 8 days after a dose of 600 r x-radiation as shown in Table IV. A

TABLE III: ULTRAVIOLET ABSORBING MATERIAL IN THE CRYPTS OF LIEBERKÜHN OF THE DUODENUM 25 HOURS AFTER APPLICATION OF 600 R X-RAYS TO THE HEAD-CHEST OF RATS

Rat No.	Extinction coefficients X-rayed rats	Control rats
7711	0.977	0.935
7712	0.766	0.857
7713	0.836	0.967
7714	0.801	0.840
7715	0.982	0.887
7716	0.967	0.840
Average	0.888	0.888

decrease was found in 4 of the 5 animals examined 10 to 17 days after x-irradiation and in 3 of 5 examined 22 to 32 days after irradiation. The data show that the effect becomes evident soon after irradiation and, with 600 r, persists for at least 17 days, after which, the limited data suggest that recovery may have been in progress.

TABLE IV: DECREASE IN ULTRAVIOLET LIGHT ABSORPTION IN THE CRYPTS OF LIEBERKÜHN IN THE DUODENUM OF THE RAT AFTER 600 R TOTAL BODY X-RADIATION

Time after irradiation	Number of rats	Average extinction coefficients X-rayed rats	Control rats	Average decrease in absorption, %
4 hrs.	5	0.766	1.013	24.4
12 hrs.	4	0.705	0.997	29.3
24 hrs.	8	0.722	1.005	28.2
2 days	2	0.846	1.304	35.1
4 days	10	0.803	0.967	17.0
8 days	11	0.732	0.855	14.4
10 to 17 days	5	0.808	0.988	18.2
22 to 32 days	5	0.796	0.821	3.1

Nature of changes.—The data shown in Tables I to IV are based on total absorption by the tissues plus refraction and scattering of light, all of which contribute to the effect on the photographic film. These data demonstrate that one or more substances in tissue are reduced by x-radiation but do not show which are reduced.

Changes in ribonucleic acid content.—Sections from the duodenum of x-radiated rats stained much less intensely with pyronin than sections from control animals. The material stainable by pyronin was removable by ribonucleinase. These facts suggest that the material was ribonucleic acid and that it was reduced in amount by x-radiation.

Ultraviolet light absorption studies yielded results which also showed that the ribonucleic acid content was reduced by x-radiation. Extinction coefficients were obtained before and after treatment by ribonucleinase. Decrease in the values of the extinction coefficients as a result of enzyme treatment of the sections was taken as a measure of the amount of ribonucleic acid originally present. The data in Table V show a marked reduction in ribonucleic acid following x-radiation at intervals from 4 hours to 4 days. A typical ultraviolet light photomicrograph of sections from x-rayed and control rats, from which the ribonucleic acid had been removed by treatment with ribonucleinase, is shown in Fig. 2.

TABLE V: COMPARISON OF AMOUNTS OF RIBONUCLEIC ACID IN THE CRYPTS OF LIEBERKÜHN IN THE DUODENUM OF X-RAYED AND CONTROL RATS. X-RAY DOSE 600 R

Rat No.	Time after irradiation	Difference in extinction coefficients before and after treatment of sections with ribonucleinase. X-rayed rats	Control rats
7371–72	4 hours	0.452	0.512
7561	12 hours	0.226	0.260
7562	"	0.318	0.463
7563	"	0.162	0.284
7549	24 hours	0.154	0.438
7552	"	0.178	0.273
7453	"	0.122	0.305
7317–21	"	0.354	0.431
7308–09	42 hours	0.402	0.468
7554–55	4 days	0.177	0.269

Changes in desoxyribonucleic acid content.—That the desoxyribonucleic acid content in the nuclei of the Lieberkühn's crypts was decreased by irradiation was shown by ultraviolet-light-absorption methods and by the Feulgen technic. The data in Table VI, obtained by the ultraviolet-light-absorption

TABLE VI: COMPARISON OF AMOUNTS OF DESOXYRIBONUCLEIC ACID IN THE CRYPTS OF LIEBERKÜHN IN THE DUODENUM OF X-RAYED AND CONTROL RATS

Rat No.	Time after irradiation	Difference between extinction coefficients obtained after ribonucleinase and after trichloracetic acid treatment X-rayed rats	Control rats
7561	12 hours	0.158	0.237
7562	"	0.138	0.418
7564	"	0.219	0.451
7549	24 hours	0.039	0.111
7550	"	0.074	0.352
7551	"	0.071	0.261
7552	"	0.267	0.360
7453–54	"	0.463	0.607
7317–21	"	0.189	0.392
7308–09	42 hours	0.077	0.090
7386–87	8 days	0.141	0.252

method, reveal that the amount of desoxyribonucleic acid was less in those sections from x-rayed than in sections from control rats. The data of Table VII, obtained by the Feulgen technic, indicate also a reduced amount in the sections from the x-rayed animals. A photomicrograph of Feulgen-stained sections of the crypts of Lieberkühn of x-rayed and control rats is illustrated in Fig. 3. The decreased amount of nuclear desoxyribonucleic acid is obvious.

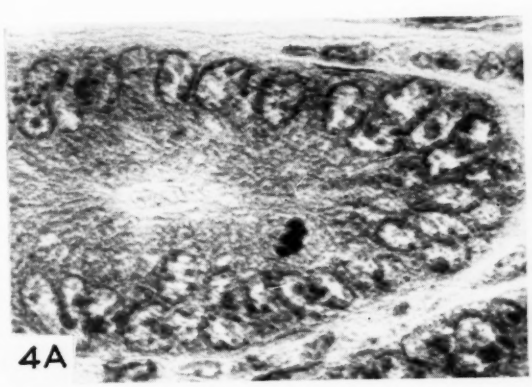
Morphological effects of 600 r total-body x-radiation on the crypts of Lieberkühn.—Photomicrographs of sections taken 4 hours after irradiation (Fig. 4) show that the amount of absorbing ma-

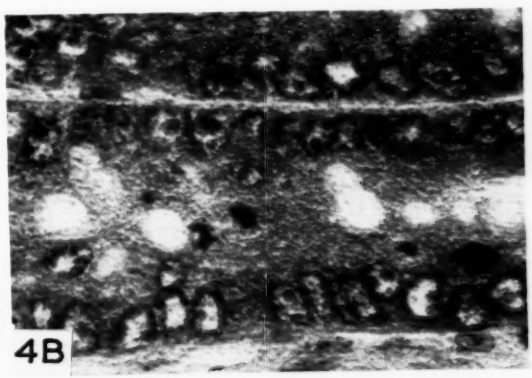
DESCRIPTION OF FIGURES 4 TO 6.

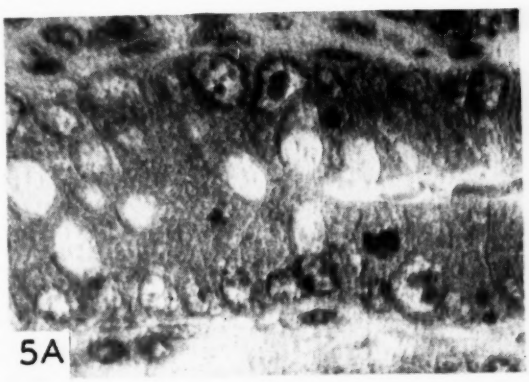
FIG. 4.—Ultraviolet light (2654 A) photomicrographs of crypts of Lieberkühn in sections from the duodenum of (A) x-rayed (600 r) and (B) control rats taken 4 hours after irradiation. Mag. × 875.

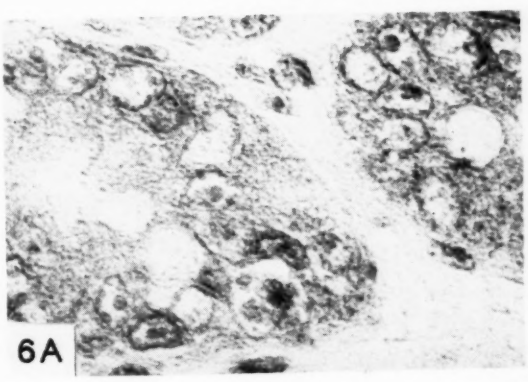
FIG. 5.—Ultraviolet light (2654 A) photomicrographs of crypts of Lieberkühn in sections of duodenum of (A) x-rayed (600 r) and (B) control rats taken 12 hours after irradiation. Mag. × 875.

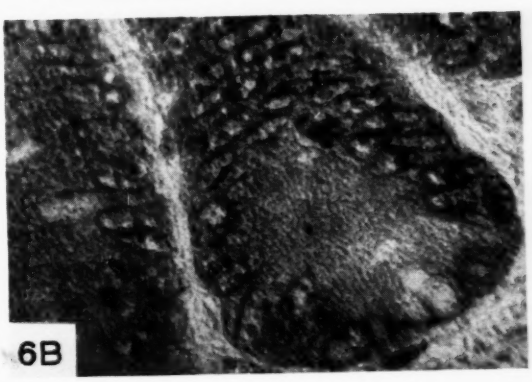
FIG. 6.—Ultraviolet light (2654 A) photomicrographs of crypts of Lieberkühn in sections from the duodenum of (A) x-rayed (600 r) and (B) control rats 24 hours after irradiation. Mag. × 875.











Figs. 4-6

terial was reduced before changes in nuclear size or decrease in cell numbers occurred. Twelve hours after irradiation, counts showed that the number of cells was reduced by approximately 40 per cent but nuclear size had not changed appreciably (Fig. 5). Twenty-four hours after irradiation, the number of cells was decreased by 50 per cent and the nuclear size was much increased (Fig. 6).

Changes in inorganic ash content.—The data of Table VIII show that the mineral ash content of microincinerated sections was less in those sections from x-rayed rats than in those sections from controls.

TABLE VII: COMPARISON OF THE DESOXYRIBONUCLEIC ACID CONTENTS OF THE CRYPTS OF LIEBERKÜHN IN THE DUODENUM OF THE RAT, AFTER 600 R X-RADIATION, DETERMINED BY THE FEULGEN TECHNIC

Rat No.	Time after irradiation	Extinction coefficients X-rayed rats	Control rats
7561	12 hours	0.893	0.910
7562	"	0.584	0.891
7563	"	0.781	1.124
7564	"	1.035	1.529
7549	24 hours	0.692	1.123
7550	"	0.803	1.245
7551	"	0.724	1.199
7552	"	1.126	1.546
7417–21	"	0.688	1.533
7453–54	"	0.891	1.308

DISCUSSION

The intense absorption of ultraviolet light by the cytoplasm in the crypts of Lieberkühn in control rats was usually so great that nuclear detail was obscure in photomicrographs. This absorbing material was shown to be ribonucleic acid or ribonucleotides, because it stained with pyronin and was removed from the tissue by a solution of crystalline ribonucleinase, an enzyme specific for ribonucleic acid. The decrease in amount of absorption by cytoplasmic ribonucleic acid was easily detected by absorption methods 4 hours after 600 r total-body irradiation. The decrease in amount was greater in 12 and 24 hours than in 4 hours. It would appear, for that reason, that the effect on the cells which caused the reduction in ribonucleic acid content was prompt and that the process of removal required some time.

A measurable reduction in the amount of nuclear desoxyribonucleic acid in the crypts of Lieberkühn occurred in 4 hours after 600 r x-radiation. As was true with ribonucleic acid, the decrease in desoxyribonucleic acid was greater in 12 and 24 hours than in 4 hours, indicative that, as with ribonucleic acid, the effect of x-radiation was prompt and that removal of this type of nucleic acid from nuclei was a function of time.

Many epithelial cells in the Lieberkühn's crypts were either killed by the radiation or died as a result of some indirect cause. Four hours after irradiation, nuclear size and the number of cells had not changed; 12 hours after irradiation, nuclear size had not changed appreciably, but the number of cells had been reduced by approximately 40 per cent; in 24 hours, nuclear size had increased and the number of cells had decreased 50 per cent. This series of events suggests that if immediate death of cells occurred after irradiation, some time was required for removal from their location in the tissue; however, it seems more reasonable to attribute cellular death to indirect causes, possibly to disruption of the metabolic ability of the cells. This could account also for the reduced amounts of nucleic acid. If synthesis of nucleic acid were inhibited, decreasing cellular amounts of these constituents would result, as that already present was utilized. Mitchell (8) suggested that x-radiation inhibited nucleic acid synthesis by cells.

Although reduction of the nucleic acid content in the cells of the crypts of Lieberkühn did not occur when the head-chest area only was irradiated, it cannot be inferred from this that the effect was directly on nucleic acid. A direct effect, as depolymerization of nucleic acid, may have occurred in view of *in vitro* experimental evidence of x-ray-induced depolymerization presented by Sparrow and Rosenfeld (10) and by Taylor, Greenstein and Hollænder (12).

TABLE VIII: COMPARISON OF THE ASH CONTENT OF THE CRYPTS OF LIEBERKÜHN AFTER 600 R X-RADIATION

Rat No. X-rayed	Rat No. Control	Time after irradiation	Densitometer readings* X-rayed rats	Control rats
7371	7372	4 hours	28.5	15.0
7373	7374	8 "	51.4	21.4
7301	7302	17 "	64.4	56.8
7303	7304	24 "	59.4	22.7
7308	7309	42 "	52.2	33.3
7280	7285	66 "	49.6	19.9
7286	7287	114 "	71.8	38.3
7386	7387	8 days	80.2	69.9

* Lower readings indicate a greater amount of ash since dark-field photomicrography was employed.

The finding of Mitchell (7), that x-radiation caused an accumulation of ribonucleotides in the cytoplasm, is not in agreement with the results reported here. The reason for the difference is not understood at present.

In agreement with the finding of decreased amounts of desoxyribonucleic acid as a result of x-radiation, are the reports of Stowell, and Langendorff and Langendorff. Stowell (11), on a limited number of observations, reported that x-radiation

of transplantable tumors produced a decrease in the amount of desoxyribonucleic acid. Langendorff and Langendorff (6) found a reduction in Feulgen stainability of chromatin following x-ray treatment.

Decreased amounts of mineral ash following x-radiation would naturally result when nucleic acids were reduced in amount, since phosphorus is a constituent of nucleic acids. Whether or not other constituents of mineral ash were reduced in amount is not known.

Reduced amounts of nucleic acids, mineral ash, and structural proteins are only end-results of a complicated series of events. The mechanism by which these results are brought about requires still further investigation.

SUMMARY

Effects of total-body x-radiation of rats on the crypts of Lieberkühn have been studied by ultraviolet-light-absorption and other methods. It was found that x-radiation caused a decrease in the amount of ultraviolet-light-absorbing material in the crypts of Lieberkühn of rats at various levels along the intestinal tract; the decrease in the amount was found at various periods of time ranging from 4 hours to 17 days. Involved in the decreased absorption of ultraviolet light were desoxyribonucleic acid, ribonucleic acid and the structural framework of the tissue, all of which were found to be reduced. The amount of inorganic ash also was found to be reduced.

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A Study of Inorganic Phosphorus Release Accompanying Glycolysis Of Blood In Cancer

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When blood undergoes glycolysis at 37° C, in addition to the loss of blood sugar, there are accompanying changes in the inorganic phosphorus. This relationship has been investigated and reported by a number of authors (2, 8, 9, 10). They agree that during the first 4 hours of glycolysis, the inorganic phosphorus of the blood in normal individuals either remains at a constant level, or is only slightly diminished. After 6 to 8 hours when glycolysis is practically complete, there is a progressive rise in the inorganic phosphorus and after the 15th hour, the concentration may reach as high as 252 mgm. per cent (6).

The inorganic phosphorus of normal blood keeps at a fairly constant level, and is elevated only in conditions such as uremia or when the calcium is greatly diminished.

Increase in the inorganic phosphorus during glycolysis is at the expense of organic soluble phosphorus of the blood cells (ester phosphorus). The explanation for the liberation of the inorganic phosphorus under these conditions, offered by Englehardt and Braunstein (3), Roche and Roche (7), and generally concurred in by most investigators, is that blood glycolysis is accompanied by a constant synthesis and hydrolysis of organic phosphate. These reactions, namely, synthesis of hexose-phosphoric acid esters as a first step in glycolysis, and secondly the hydrolysis of these esters are interrelated, although the hydrolysis may proceed independently of the synthesis. When hydrolysis exceeds synthesis inorganic phosphorus is liberated.

Glycolysis can be inhibited by the introduction of sodium fluoride as an anti-coagulant. When blood taken in fluoride was incubated for 6 hours at 37° C, a release of inorganic phosphorus could be demonstrated, in spite of the fact that no glycolysis had occurred.

The results of the determinations of glucose and inorganic phosphorus made before incubation and at 6 hours and 24 hours after incubation using fluoride and heparin as anti-coagulants, are given in Table I. A comparison of the inorganic phosphorus values at the end of the 24 hours incubation period shows a greater release of inorganic phorphorus in those blood specimens subjected to glycolysis.

Inhibition of glycolysis can also be induced by inactivation of heparinized blood at 57° C, for 30 minutes. Accompanying this inactivation there is also a release of inorganic phosphorus. In a series of 7 cases the increase ranged from 0.7 to 3.7 mgm. per cent. This is apparently the release of a heat labile phosphorus fraction.

At the end of the inactivation period, the blood samples were placed in an incubator at 37° C. for 6 hours. Glucose values showed no change during this period, but there was a further increase in the inorganic phosphorus of from 1.0 to 4.4 mgm. per cent. It is apparent that at least two different enzymatic processes are involved in the release of inorganic phosphorus. One, in which glycolysis plays the major role, and another which proceeds independently of glycolysis.

During a study of the role of glycolysis of the blood in cancer (to be published), observations were made at the same time on the release of inorganic phosphorus. As far as we are aware, no investigations of the rate of hydrolysis of phosphate esters during glycolysis in cancerous individuals have been reported. In view of this, we have made a study of the inorganic phosphorus during glycolysis in 196 individuals of whom 64 were cancerous and 132 were noncancerous.

PROCEDURE

Blood was obtained by veni-puncture after an over-night fast. A solution of heparin[1] (Lederle, 100 mgm. per 1 ml.) was used as an anti-coagulant in the amount of 0.1 ml. per 10 ml. of blood. Immediately after withdrawal of the blood, the inorganic phosphorus was determined by the method of Fiske and Subbarow (5) with a Klett-Summerson colorimeter. The remaining blood was kept in a closed container and placed in an incubator at 37° C. for a period of 6 hours. This time period was chosen because glycolysis was then practically

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[1]Our analysis of the heparin solution gave negative results for inorganic phosphorus.

completed. Inorganic phosphorus was again determined on this incubated specimen. The glucose was determined by the method of Benedict (1).

A loss of 70 per cent or more of blood sugar from the initial blood sugar value, in 4 hours incubation at 37° C., was considered to be an accelerated glycolytic rate.

Normal controls.—In 25 normal individuals, the increase in inorganic phosphorus varied between —2.1 and +3.2 mgm. per cent, after 6 hours' incubation, wth a mean value of 0.93 mgm. per cent.

Cancer cases.—In 64 individuals suffering from some form of neoplastic malignant disease, the increment of inorganic phosphorus after 6 hours of glycolysis varied between —1.8 and +5.6 mgm. per cent, with a mean value of 2.22 mgm. per cent.

Non-cancer cases.—In 107 patients free from neoplastic disease, the inorganic phosphorus increment varied between —1.3 and 6.4 mgm. per cent, with a mean value of 1.53 mgm. per cent.

Evaluation of the mean results of each group, by the statistical method of Fischer (4), shows that the *P* value between the cancerous group and the normal group is 0.001, the *P* value between the cancerous group and the non-cancerous group is 0.016 and the *P* value between the normal group and noncancerous group is 0.136. *P* represents the frequency with which the difference between two means may be due to chance alone. When the *P* value is 0.05 or less, the difference between the means is statistically significant. We have therefore adopted an increase of more than 1.53 mgm. per cent as a significant rise.

If we consider all of the 196 cases reported regardless of diagnosis from the standpoint of the mean value of 1.53 mgm. per cent or more, in the inorganic phosphorus, we find that 96 showed the increase, while 101 did not. A breakdown of the data into cancerous and noncancerous groups, shows that in 46 of 64 cancer patients (72 per cent), there was an increase of inorganic phosphorus greater than 1.53 mgm. per cent, while of the 132 noncancerous patients studied, only 50 or (38 per cent), showed a similar increase.

Table II records the results of our observations on 132 individuals who were free from neoplastic malignant disease. The glycolytic rate is shown concomitantly with the increase in inorganic phosphorus. If we consider the release of inorganic phosphorus in conjunction with the glycolytic rate, we find that in 47 of 49 cases showing a rise greater than 1.53 mgm. per cent, there was also an acceleration of the glycolytic rate of 70 per cent or more. In 29 cases in which there was also an acceleration of the glycolytic rate, the phosphorus release was under 1.53 mgm. per cent. In the 54 instances in

TABLE I: EFFECT OF GLYCOLYSIS ON INORGANIC PHOSPHORUS RELEASE

Specimen No.	Before incubation				6 hours after incubation				24 hours after incubation			
	Glucose		Inorg. P.		Glucose		Inorg. P.		Glucose		Inorg. P.	
	H*	F*	H	F	H	F	H	F	H	F	H	F
1	93	86	2.6	2.8	5	89	4.3	5.6	5	89	23.1	6.5
2	91	91	3.1	3.0	27	89	4.7	5.9	3	89	26.0	8.8
3	83	84	4.5	4.5	4	83	7.0	7.4	4	83	24.0	9.0
4	98	99	4.2	4.0	4	85	9.5	4.8	4	105	23.2	6.4
5	80	79	4.7	4.2	19	80	6.6	6.6	15	80	23.4	8.8

* H—Heparin, F—Fluoride, (all values given in mgm. per cent)

TABLE II: CHANGES IN INORGANIC PHOSPHORUS OF THE BLOOD ACCOMPANYING GLYCOLYSIS IN NORMAL AND PREGNANT INDIVIDUALS, AND IN PATIENTS WITH NONMALIGNANT DISEASES

Diagnosis	No. of cases	Cases showing an increase greater than 1.53 mgm./%	Cases with glycolysis		Cases showing an increase less than 1.53 mgm./%	Cases with glycolysis	
			under 70%	over 70%		under 70%	over 70%
Normals	25	8	0	8	17	15	2
Pregnancy	4	4	0	4	0	0	0
G. I. ulcers	13	8	0	8	5	2	3
Liver diseases	7	2	0	2	5	3	2
Hypertension and cardio-vascular disease	15	1	0	1	14	8	6
Thyroid disturbances	5	2	0	2	3	3	0
Benign growths	19	7	1	6	12	8	4
Diabetes	6	0	0	0	6	5	1
Bacterial disease, acute	16	10	0	10	6	3	3
Bacterial disease, chronic	13	4	1	3	9	4	5
Miscellaneous	9	3	0	3	6	3	3
Totals	132	49	2	47	83	54	29

TABLE III: CHANGES IN THE INORGANIC PHOSPHORUS OF THE BLOOD ACCOMPANYING GLYCOLYSIS IN PATIENTS WITH MALIGNANT TUMORS

Organs involved	No. of cases	Cases showing an increase greater than 1.53 mgm./%	Cases with glycolysis		Cases showing an increase less than 1.53 mgm./%	Cases with glycolysis	
			under 70%	over 70%		under 70%	over 70%
Respiratory system	6	4	1	3	2	2	0
Gastro-intestinal system	31	23	7	16	8	7	1
Genito-urinary system	14	11	4	7	3	3	0
Breast	5	2	2	0	3	3	0
Miscellaneous	8	6	1	5	2	2	0
Totals	64	46	15	31	18	17	1

which the glycolytic rate was under 70 per cent, the phosphorus release was less than 1.53 mgm. per cent.

The data given in Table III shows the results in 64 individuals with neoplastic malignant disease. Of these, in 46 cases in which a phosphorus release greater than 1.53 mgm. per cent was observed, 15 did not have an accelerated glycolytic rate. Only 1 patient with a glycolytic rate of over 70 per cent, showed a phosphorus release lower than 1.53 mgm. per cent. In the other 17 cases in which the glycolytic rate was under 70 per cent, the phosphorus release was less than 1.53 mgm. per cent.

CONCLUSIONS

It has been shown that phosphorus release in the blood may occur with or without glycolysis; however glycolysis seems to influence the rate of liberation.

Liberation of inorganic phosphorus from the blood, (after glycolysis is complete), has been studied in 196 individuals, of whom 64 were suffering from some form of malignant neoplasm. The blood of those patients having a malignant tumor shows a greater tendency towards an increased liberation of inorganic phosphorus. The increase is not entirely due to glycolysis.

A comparison of the mean value for the inorganic phosphorus increase of the blood, between patients with malignant and nonmalignant conditions, is statistically significant.

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Abstracts

Reports of Research

The Chemistry of Carcinogenic Nitrogen Compounds. New Derivatives of the Angular Benzacridines and of Some Related Nuclei. Buu-Hoï, Hg. Ph. [Lab. of Organic Chem., École Polytechnique, Paris, France] *J. Chem. Soc. London*, 792-795. 1946.

Several methyl and halogen derivatives of 1,2- and 3,4-benzacridine have been synthesized for tests for carcinogenic action. Recent work by this author and others (*Compt. rend. Soc. de biol.*, **139**:955. 1945) showed that 5,8-dimethyl-1:2-benzacridine had carcinogenic action on the skin of the mouse of the same order as that of 20-methylcholanthrene. The isomeric 5,7- and 5,8-dimethyl-3,4-benzacridines have little or no such action. A series of *N*-alkyl-1:2-bencarbazoles was synthestized; none of these appear to be actively carcinogenic. The new compounds described are being examined also for anti-carcinogenic action.—E.L.K.

The Effect of Steric Hindrance on the Course of Pfitzinger Reactions. Buu-Hoï, Ng. Ph. Lab. of Organic Chem., École Polytechnique, Paris, France] *J. Chem. Soc. London*, 795-797. 1946.

The synthesis of 10-propionyl-3,4-benzpyrene from 3,4-benzpyrene is described.—E.L.K.

Tumors Induced in Mice with *p*-Diazoaminobenzene. Kirby, A. H. M. [Glasgow Roy. Cancer Hosp., Glasgow, Scotland] *Cancer Research*, 7:000-000. 1947.

The azo dye intermediate, diazoaminobenzene (DAAB), was found not to cause lesions of the forestomach in stock mice fed this substance in either a stock diet or a restricted diet known to favor hepatic tumor formation due to azo dyes. Similarly, subcutaneous injections of DAAB in peanut oil failed to yield any tumors at the site of injection, or elsewhere, in similar mice. Stock mice painted successively with 0.5, 1.0, 2.0 and 5.0% solutions of DAAB in acetone developed hyperkeratotic growths at the site of painting; in a male mouse surviving 346 days and in a female surviving 601 days, squamous carcinomas were induced. DAAB is thus a carcinogen for the skin of the mouse. Toxic damage to kidneys and hyaline degeneration of spleen, kidney and liver were the only notable lesions found in internal organs of any of the mice used in these experiments. The nature of the lesions, both those found and those absent, would indicate that DAAB is not converted *in vivo* by the mouse into *p*-aminoazobenzene, a process easily occurring *in vitro*.—Author's abstract.

On the Fate of Carcinogenic Hydrocarbons in the Animal Body. Larionow, L. Th. [Byelorussian State Med. Inst., Minsk, B.S.S.R.] *Cancer Research*, 7:000-000. 1947.

The author has studied certain questions connected with the distribution and fate of 3,4-benzpyrene in the animal organism. For this purpose benzene extracts were made of the organs and fluids of the body and their fluorescence studied by spectraphotography. It has been found that benzpyrene injected into the blood of mice and rats rapidly disappears from the blood stream, but is retained by the lungs, the liver and adipose tissues, as well as by the walls of the intestine. A flourescent benzpyrene derivative is excreted in the urine of mice and rabbits, its fluorescence spectrum being identical to that excreted in bile. Benzpyrene injected subcutaneously is also retained by adipose tissues, the liver and the intestine. The cerebrospinal fluid of rabbits and dogs inoculated intravenously with small quantities of benzpyrene was found not to contain any of the hydrocarbon; nor was benzpyrene observed in the milk of either rabbits or dogs that were suckling their young. In dogs affected with experimental hepatitis and fatty degeneration of the liver, the period of benzpyrene elimination with the bile is lengthened. The greater part of benzpyrene or methylcholanthrene administered to mice and rats with their food is excreted in the feces in an unchanged state. Part of the hydrocarbon is absorbed and some is retained by the wall of the forestomach. The paper is illustrated by a number of photographs of spectra.—Author's abstract.

Induction of Ovarian Tumors in Mice by X-Rays. Furth, J., and Boon, M. C. [Cornell Univ. Med. Coll., New York Hosp., New York, N. Y.] *Cancer Research*, 7:000-000. 1947.

Following irradiation of 4 to 6 weeks old mice with 87 r, 175 r, or 350 r, ovarian tumors began to appear when the mice were about 11 months of age. The frequency of these neoplasms increased with time and almost every mouse that lived to be 17 months old developed a unilateral or bilateral ovarian growth irrespective of the dose of irradiation.

These ovarian growths are compared as to pathogenesis and autonomous character, with the hyperplastic nodules that result from implantation of normal ovaries into the spleens of castrated mice, as described by Biskind and Biskind. The conclusion is reached that the latter are not autonomous growths (although they sometimes give rise to true neoplasms); while the x-ray-induced ovarian growths, on the contrary, are readily transplantable autonomous growths.—Authors' abstract.

The Effect of Castration and Sex Hormones upon the Incidence of Lymphomatosis in Chickens. Burmester, B. R., and Nelson, N. M. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Poultry Science*, **24**: 509-515. 1945.

Three hundred and sixty-eight White Leghorn chickens of both sexes were used to determine the effect of castra-

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tion and implantation of diethylstilbestrol and testosterone propionate upon the incidence of lymphomatosis.

Castrated males, whether inoculated with blood of donors having lesions of lymphomatosis or non-inoculated, had a significantly higher incidence of lymphomatosis than normal males of the same breeding. Castrated females also had a slightly higher percentage of lymphomatosis than the normal controls. These differences among the females, however, were not significant. Capons treated with female hormone had a significantly lower incidence than untreated capons although no significant effect was demonstrated on normal males. Males and capons treated with the male hormone had a significantly lower incidence of lymphomatosis than untreated males and capons. The results obtained suggest that the male hormone increases the resistance of chickens to lymphomatosis and this in part probably accounts for the fact that the incidence of this disease is usually lower among males than among females.—B.R.B.

Natural Transmission of Avian-Lymphomatosis. WATERS, N. F. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Poultry Science*, **24**:226-233. 1945.

This report involves approximately 4,700 White Leghorn chickens, which were divided into groups and subjected to different environmental modifications in an attempt to measure the influence of such environment on the natural transmission of lymphomatosis. Lymphomatosis was present in chickens under 4 months of age despite the fact that direct contact with previously infected birds did not exist. Evidence is presented to show that lymphomatosis is transmitted both by way of the egg and by contact with infected birds. Careful selection and testing of certain families hatched and reared in isolation and maintained under quarantine prevented the occurrence of lymphomatosis to 700 days of age. The hatching and rearing of chickens in isolation, even though placed in an environment where sanitary practices or quarantine measures exist, did not prevent the occurrence of lymphomatosis unless the parents of such birds were free of the disease.—B.R.B.

Types of Lymphomatosis among Different Inbred Lines of Chickens. WATERS, N. F., and PRICKETT, C. O. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Poultry Science*, **25**:501-508. 1946.

The chickens used in this study were 3257 Single Comb White Leghorns maintained to 600 days of age and represent 6 years of selective inbreeding among 14 different lines for resistance or susceptibility to lymphomatosis. None of the birds were inoculated and the report is based on females only. All chickens were confined in buildings throughout life and were exposed more or less to the same environment. Necropsies on all of the chickens involved, showed that 24.4 % had the visceral form, 9.8% had the neural form, and 1.0% had the occular form of lymphomatosis. Neural manifestations although occurring at all age periods were most prevalent during the first 300 days of age. Visceral manifestations were observed after 30 days of age and increased in frequency after this period. Certain of the inbred lines showed a high early incidence of neural lymphomatosis while other lines showed a high incidence of the visceral form with little or no occurrence of the neural form. This marked variation in the percentage of various forms of lymphomatosis among the several inbred lines was interpreted as an indication of an organ specificity to this disease which can be influenced by genetic selection.—B.R.B.

The Genetic Relationship between Mortality from Induced and Spontaneous Lymphomatosis. HEISDORF, A. J., BREWER, N. R., and LAMOREUX, W.F. [Kimber Poultry Breeding Farm, Niles, Calif.] *Poultry Science*, **26**;67-73. 1947.

Subcutaneous inoculation with lymphomatous tissue did not differentiate between a line of White Leghorn chickens selected for resistance to naturally occurring lymphomatosis and another line selected for susceptibility. When the inoculum was placed in the crop, eyes, and nostrils of chicks a significant difference in the incidence of lymphomatosis between the 2 selected lines was obtained among those maintained for 225 days.

There was no significant relationship between losses from lymphomatosis among families of birds that had been inoculated subcutaneously and losses suffered by their full or half sibs which were raised as controls under natural exposure; however, when the inoculum was administered by the oral route and the chickens maintained for 225 days a significant correlation with sibs raised under natural exposure was obtained.—B.R.B.

Breeding for Resistance and Susceptibility to Avian Lymphomatosis. WATERS, N. F. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Poultry Science*, **24**: 259-269. 1945.

This study reports the findings for 5 years of selective inbreeding in chickens for resistance and for susceptibility to lymphomatosis. The foundation chickens used in this report were all White Leghorns and were introduced as hatching eggs originating from widely separated geographic regions. All breeding operations have been limited to selection within the original chickens or their descendants. At the end of a 600 day period for the first 4 generations about 60% of all birds were dead. Lymphomatosis accounted for approximately half of this loss. The remainder included losses from cannibalism, reproductive disorders, coccidiosis, and minor physiological disturbances. The percentage of losses at 30 day intervals, based on lymphomatosis or other causes, for all years are strikingly similar for both groups. In the absence of all other diseases, except coccidiosis, this similarity suggests that death in these 2 groups may be associated. By means of selective inbreeding within 15 lines of chickens it has been possible to show progressive increases and decreases in the incidence of lymphomatosis. These increases and decreases are believed to be due to the direct influence of genetic selection, and represent a definite segregation of genes for resistance or for susceptibility to lymphomatosis.—B.R.B.

The Development of Strains of White Leghorns Genetically Resistant to Lymphomatosis. HUTT, F. B., and COLE, R. K. [Cornell Univ., Ithaca, N. Y.] *Genetics*, **32**:91-92. 1947.

Beginning with an unselected population in 1935, 3 strains of White Leghorns have been differentiated by selection, 2 of which are strikingly resistant, the other extremely susceptible. Diallele and reciprocal crosses for 2 years proved that the difference between these resistant and susceptible strains is genetic and not attributable to any cytoplasmic transmission through the egg of passive immunity in the resistant lines, or of the causative agent in the susceptible one.—G.W.W.

Lymphomatosis in Chickens as Influenced by Diallel Crossing. WATERS, N. F. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Poultry Science*, **24**: 387-390. 1945.

A study was made by means of diallel crossing of the influence of the sire on lymphomatosis. The data include 12 sires and 18 dams of the Single Comb White Leghorn breed. In all, 6 sets of dams, consisting of from 2 to 4 dams in each set, were each mated to 2 different sires, 387 progeny being produced. An analysis of these data, as they refer to the incidence of lymphomatosis with relationship to other mortality as well as age at death, shows that the progeny from one sire differs significantly in several sets from those of another when both are mated to the same dams. These results were interpreted to suggest that the differences were due largely to sire influence.—B.R.B.

The Incidence of Lymphomatosis Among Male and Female Chickens. BURMESTER, B. R. [U. S. Regional Poultry Research Lab., East Lansing, Mich.] *Poultry Science*, **24**:469-472. 1945.

The incidence of lymphomatosis to 300 days of age based on gross findings at necropsy, among 701 male and 1,070 female chickens of the first population raised at the Regional Poultry Research Laboratory was analyzed and the results presented. It was found that the occurrence of this disease in noninoculated females, whether raised in isolation or in contact with inoculated birds, was about twice that in similarly treated males. The difference was reduced to insignificance when birds were inoculated intravenously with blood of birds having lymphomatosis. Ovarian involvement occurred more than three times as frequently as testicular involvement regardless of inoculation, however, this did not entirely account for the difference between the sexes in the noninoculated groups.—B.R.B.

Protein-Chemical Aspects of Cancer. TOENNIES, G. [Lankenau Hosp. Research Inst., and Inst. for Cancer Research, Philadelphia, Pa.] *Cancer Research*, **7**:000-000. 1947.

In this review a synopsis is attempted of the chemical literature (up to and including 1945) concerned with the question of whether malignant growth is associated with specific changes in protein composition. Related investigations are critically compared and enough detail from the original papers (some 200) is reproduced to make possible some evaluation of validity and significance. Proteins and nucleic acids as well as their respective component parts and building stones are considered. Except for aspects bearing directly on protein composition, enzymology has been excluded. In addition to the protein chemistry of cancer tissue, that of noncancerous organs of cancer bearers (including tissues and blood) and of excretory products is reviewed. From the assembled data no factual conclusions are derived because of the great disparity between problems raised and results established. However, the inference is drawn that the experimental attempts of the past have largely served to reveal the technical complexity of the problem. The more important aspects of the difficulties encountered in this field are briefly characterized under the following headings: (a) homologous tissue, (b) homogeneous tissue, (c) deterioration of tissue, (d) disintegration of tissue, (e) structural complexity of cells, and (f) dynamics of metabolism.—Author's abstract.

Leukoagglutination as a Serological Diagnosis for Avian Lymphomatosis. KISSLING, R. E. [Ohio State Univ., Columbus, Ohio.] *Poultry Science*, **26**:74-77. 1947.

In an attempt to develop a serological test for the diagnosis of avian lymphomatosis, a total of 94 chicken sera was examined for the presence of lymphocyte agglutinins. The antigen consisted of washed, stained, canine lymphocytes. The test was performed as a rapid plate agglutination method at p ^{H7} and a temperature of 37° C. Forty-eight sera showed the presence of lymphocyte agglutinins; of these 83.3% had demonstrable lesions of the leukosis complex. Of the nonreacting birds none had gross lesions of lymphomatosis whereas 2 showed microscopic lesions. Both of the latter birds were less than 3 months of age. There appeared to be no cross agglutination with other disease entities.—B.R.B.